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## SACROCOCCYGEAL TUMORS

ADENOCARCINOMA OF A CYSTIC CONGENITAL EMBRYONAL REMNANT

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The purpose of this paper is to introduce briefly the subject of congenital sacrococcygeal tumors, and to report a case of carcinoma that appears to have arisen from remnants of postanal intestine.

The general heading "sacrococcygeal tumors" includes a great variety, from simple fibromas and lipomas at one extreme to parasitic fetuses at the other. Dermoid cysts, teratomas and teratoid tumors are included, as well as simpler tumors, both solid and cystic.<sup>1</sup> The one feature common to practically all tumors that occur in this region is their congenital origin. Some occupy the anterior<sup>2</sup> and some the posterior<sup>3</sup> surface of the sacrum. In other cases, the main bulk of the tumor is slung under the coccyx, while portions of it lie both anterior and posterior to the sacrum.<sup>4</sup> Some of these tumors are derived from embryonal structures that normally undergo more or less complete obliteration.<sup>5</sup> Some of these show one type of tissue only, such as tissue of the nervous system or of the intestinal tract, while "others are complex, difficult of interpretation, and seem to involve more than one embryonal structure" (Ewing<sup>1c</sup>). The latter are referred to as teratoid tumors,<sup>1</sup> thus distinguishing them from teratoma, which contains structures or tissues that cannot be derived from local embryonal structures.

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From the Pathological Laboratory, St. Joseph's Hospital.

Presented before the Section of Pathology, Academy of Medicine, Toronto, Ontario, March 24, 1931.

1. (a) Borst, Max: *Centralbl. f. allg. Path. u. path. Anat.* **9**:449, 1898. (b) Bland-Sutton, John: *Tumors Innocent and Malignant*, London, Cassell & Co., Ltd., 1922, p. 490. (c) Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1928, p. 1033.

2. Hundling, H. W.: *Surg., Gynec. & Obst.* **38**:518, 1924. Borst.<sup>1a</sup> Bland-Sutton.<sup>1b</sup>

3. Mallory, F. B.: *J. M. Research* **131**:113, 1904-1905. Cutler, G. D.: *Surg., Gynec. & Obst.* **37**:779, 1923. von Bergmann, E.: *Berl. klin. Wchnschr.* **21**:761, 1884. Borst.<sup>1a</sup> Bland-Sutton.<sup>1b</sup>

4. Freyer, M.: *Virchows Arch. f. path. Anat.* **58**:509, 1873. Jastreboff, N.: *ibid.* **99**:500, 1885. Simpson, J. K.: *J. A. M. A.* **84**:139, 1925.

5. (a) Mallory, F. B.: *Am. J. M. Sc.* **103**:263, 1892. (b) Hermann, C., and Tourneux, F., quoted by Mallory, Borst<sup>1a</sup>, Hundling<sup>2</sup> and others. Borst<sup>1a</sup> Bland-Sutton.<sup>1b</sup> Ewing.<sup>1c</sup> Hundling.<sup>2</sup>

Sacrococcygeal tumors are frequently noted at birth, but sometimes escape notice until adult life. A relatively small proportion appear to become malignant.<sup>6</sup> The embryonal structures from which they may be derived include remnants of neural tube, which may be present anywhere from the lower end of the sacral canal to the tip of the coccyx,<sup>7</sup> neurenteric canal and postanal intestine,<sup>8</sup> remnants of which, when present, are usually situated on the anterior surface of the sacrum, possibly the supernumerary coccygeal vertebrae normally present in the early stages of the embryo<sup>9</sup> and notochord.<sup>10</sup>

Possibly the first recorded congenital sacrococcygeal tumor was that described by Peu, a French obstetrician of the seventeenth century (Hennig<sup>10b</sup>). Hamant, Cornil and Mosinger<sup>6h</sup> collected three hundred cases of sacrococcygeal tumors in the literature up to 1929. Most of the sacrococcygeal tumors discovered at birth or during infancy are teratomas or teratoid tumors. They are frequently large enough to cause dystocia. Speaking of teratoid tumors, Ewing<sup>1c</sup> said, "One-third of the subjects are born dead, and 90 per cent. of the others die in the first few days." Duncan<sup>11</sup> referred to a series of collected cases of congenital sacral tumors showing 50 per cent mortality and 50 per cent recovery following operation during the first year of life, and approximately 78 per cent complete recovery, 14 per cent partial recovery and 7 per cent mortality following operation after the first year of life. One of the component tissues of a teratoma or a teratoid tumor may become malignant during infancy.<sup>12</sup> In some cases in which malignancy had apparently not been suspected, recurrence with metastases has followed removal (Hinterstoisser's case, cited by Parin<sup>6a</sup>).

Sacrococcygeal tumors discovered later in life are usually less complicated. They include dermoids, simpler cystic tumors lined with squamous or columnar epithelium, gliomas, ependymal cell tumors and

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6. (a) Parin, reviewed by Galletly, A.: *Proc. Roy. Soc. Med.* **17**:105 (pt. 3) 1923-1924. (b) Stewart, J. D.; Alter, N. M., and Craig, J. D.: *Surg., Gynec. & Obst.* **50**:85, 1930. (c) Kraske, P.: *Samml. klin. Vortr.*, 1897, nos. 183 and 184. (d) Rostock, P.: *Virchows Arch. f. path. Anat.* **267**:352, 1928. (e) Heusner, R.: *Centralbl. f. allg. Path. u. path. Anat.* **24**:1025, 1913. (f) Moersch, F. P.: *M. Clin. North America* **10**:715, 1926. (g) Pringle, S.: *Lancet* **1**:1643, 1907. (h) Hamant, A.; Cornil, L., and Mosinger, M.: *Ann. d'anat. path.* **6**:1224, 1929.

7. Mallory.<sup>5a</sup> Hermann and Tourneaux.<sup>5b</sup>

8. Peyron, A.: *Bull. Assoc. franç. p. l'étude du cancer* **17**:613, 1928; reviewed by Dukes, C. E.: *Cancer Rev.* **5**:129, 1930. Borst.<sup>1a</sup> Bland-Sutton.<sup>1b</sup> Ewing.<sup>1c</sup>

9. Keith, A.: *Human Embryology and Morphology*, London, Edward Arnold & Co., 1923, p. 65. Borst.<sup>1a</sup>

10. (a) Pandalia, K. G.; Forsyth, W. L., and Stewart, M. J.: *J. Path. & Bact.* **27**:139, 1924. (b) Hennig, L.: *Beitr. z. path. Anat. u. z. allg. Path.* **28**:593, 1900. Ewing.<sup>1c</sup> Moersch.<sup>6f</sup> Hamant et al.<sup>6h</sup>

11. Duncan, H. A.: *M. J. & Rec. (supp.)* **120**:108, 1924.

12. Parin.<sup>6a</sup> Stewart et al.<sup>6b</sup>

chordomas. Galletly<sup>13</sup> held that all sacrococcygeal tumors discovered at birth are teratomas, while those not discovered until puberty or later are derived from embryonal remnants (dermoids excepted). There appear to be exceptions to the rule that sacrococcygeal tumors discovered at birth are teratomas or teratoid tumors (case of Fletcher and Waring,<sup>14</sup> cited under "Comment"). On the other hand, a complicated teratoid tumor may occasionally be found in an adult beyond middle life, and such a tumor may become malignant (Czerny's case, cited by Parin<sup>6a</sup>). Discovery in adults is made by accident or as the result of the onset of the symptoms due to the assumption of growth (sometimes malignant growth) by a previously resting structure or tissue. Frequently the symptoms consist of "sciatic pains."<sup>15</sup> In Moersch's<sup>6c</sup> series of presacral tumors, 83 per cent could be felt by rectum, and 37 per cent showed roentgenographic evidence of destruction of bone in the sacral region. Malignant sacrococcygeal tumors in adults have been classified as ependymal cell tumors ("carcinomas"), chordomas, sarcomas, carcinomas of postanal intestine and carcinomas of doubtful origin. Malignancy may supervene in dermoids. Gaffa collected a number of such cases (Duncan<sup>11</sup>).

#### REPORT OF A CASE

*History.*—A married woman, age 38, the mother of three children, was admitted to St. Joseph's Hospital, on March 7, 1930, complaining of continual pain, or rather discomfort, in the left buttock ("cannot sit down"). On standing, she felt a weight and a pulling sensation. Nearly two years before, she first noticed that she could not sit for any length of time with comfort. About fifteen months before, distress became more noticeable. At that time a doctor examined her, but found no cause for the distress. About a month before admission, she was examined by her physician, who discovered and tapped a cyst at the lower end of the spine, from which he removed about a pint (473 cc.) of fluid.

The family history is not relevant. The only points in the past and personal history that have any bearing on the case are that the first confinement was difficult, and that the present complaint started not long after the birth of the last child.<sup>16</sup>

*Examination and Operation.*—Physical examination showed nothing abnormal, except puffiness over the sacrum, due to a cyst situated in that region. A roentgenogram (fig. 1) showed a deficiency involving the lower end of the sacrum and the coccyx. The preoperative diagnosis was: dermoid of the coccygeal region.

At operation, March 7, a cyst was found, which occupied a deficiency in the sacrum and coccyx, and lay against the wall of the rectum anteriorly and immediately beneath the skin posteriorly. It was not adherent to the skin, but was adherent to the posterior wall of the rectum at one point. The cyst was drained of about a

13. Galletly, A.: Proc. Roy. Soc. Med. **17**:105 (pt. 3) 1923-1924.

14. Fletcher, H. M., and Waring, H. J.: Tr. Path. Soc. London, 1900, p. 226.

15. Hundling.<sup>2</sup> Moersch.<sup>6c</sup>

16. Hundling<sup>2</sup> also reported a case of presacral carcinoma in which there was a history of difficult labor with the patient's first child.



pint (473 cc.) of thick material that "looked somewhat like pus." The sac was excised and the cavity packed with gauze.

The gross specimen consisted of a number of thin sheets resembling fibrous tissue, one surface being brownish pink and finely granular.

*Histology of Tumor.*—Figure 2 shows the cryptlike or intestinal glandlike arrangement of the tissue lining the inner surface of the cyst. It also shows the columnar type of epithelium, which resembled intestinal epithelium. In places



Fig. 1.—Roentgenogram showing deficiency in the sacrum and coccyx. It is not certain whether the vertebra marked *x* is a fused last lumbar vertebra or the first sacral vertebra. If it is a fused last lumbar vertebra, there are three sacral vertebrae and the deficiency; but if it is counted as the first sacral vertebra, there are four sacral vertebrae and the deficiency. Dr. L. R. Hess reported the deficiency as erosion. Both congenital deficiency and erosion occur with sacrococcygeal tumors. The sacrum has been found rudimentary in congenital tumors on the ventral surface of the sacrum by Pannwitz (quoted by Borst <sup>1a</sup>), Stewart, Alter and Craig,<sup>6b</sup> Pandalia, Forsyth and Stewart <sup>10a</sup> and others. On the other hand, Hundling <sup>2</sup> and others referred to the presence of erosion in such cases. In the present case, the gradual onset of symptoms of pain and discomfort on sitting was probably associated with the progress of the erosive process. The pain was on the left, the side with the long projecting remnant of bone. It is impossible to determine whether a congenital deformity did or did not exist previous to the onset of erosion.

where the epithelium covered the rounded surface between cryptlike depressions, the cells were elongated and assumed a rather fan-shaped arrangement, such as is frequently seen on the rounded surface between crypts in sections of appendix. The stroma contained plasma cells, lymphocytes and eosinophils in places. A higher magnification of the outlined area is shown in figure 3.

Figure 3 is shown particularly for the sake of the goblet cells that were present. By direct observation of this field under the microscope, eight goblet cells could be seen. Only five are distinctly within the focal plane in figure 3. The one that is sharply in focus shows its nucleus. To the left of this goblet cell are two mitotic figures. Basic-staining material like mucus was observed in the crypts or lumina of the glands in some of the sections.



Fig. 2.—Tissue forming part of the lining of the cyst. It shows resemblance to intestinal glands and stroma. A higher magnification of the area encircled is shown in figure 3.

In the section from which figure 4 is taken there was at one point a sudden transition from tissue having the comparatively orderly arrangement seen in figures 2 and 3 to frankly carcinomatous tissue. This reminded one very much of the sudden transition that is so frequently seen from normal intestinal mucosa to adenocarcinoma, except that the glandular character of the carcinoma in the present instance was much less obvious and the histologic evidence of malignancy was much greater. Lumina were minute and difficult of detection. In parts of this microscopic section, however, and also in other sections, the adenomatous character of the malignant tissue was evident. This photomicrographic field was chosen primarily to demonstrate the histologically malignant characteristics of the tissue. Other sections showed widespread infiltration of the fibrous wall of the cyst by tissue of this frankly carcinomatous type, usually with a distinct attempt at a gland-like arrangement. There were some extremely large cells, with highly hyperchromatic nuclei.

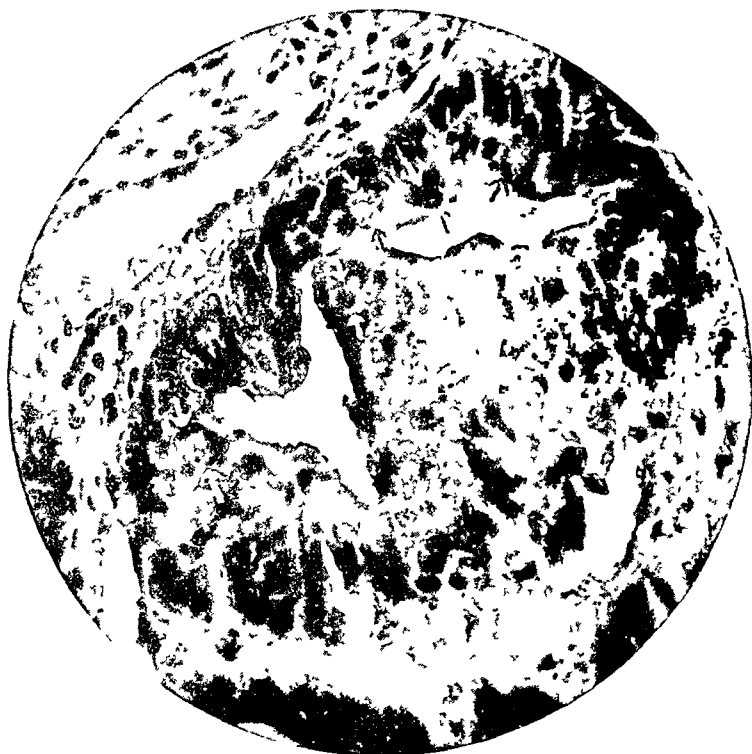


Fig. 3.—Higher magnification of the area outlined in figure 2, showing goblet cells among the columnar epithelial cells of two glands.

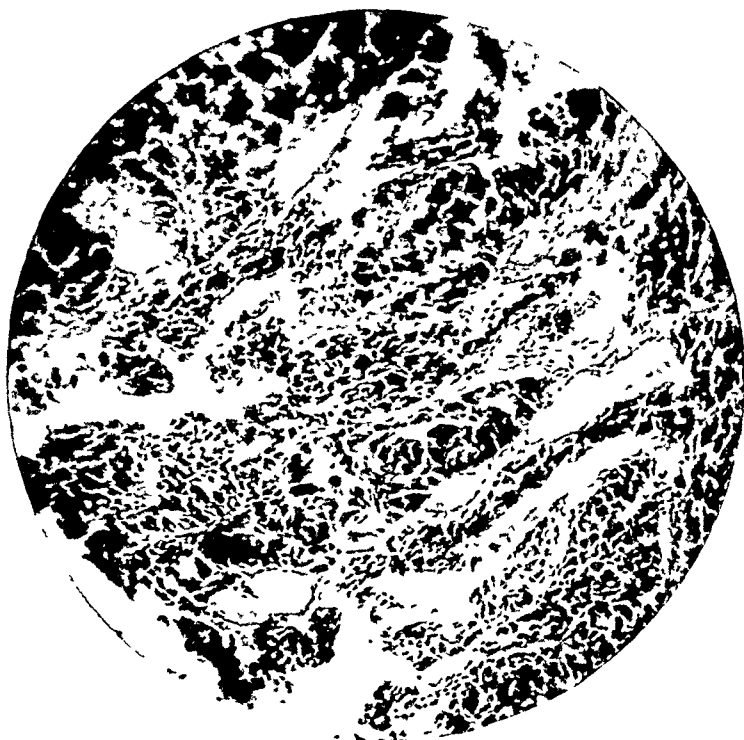


Fig. 4.—Adenocarcinomatous tissue forming part of the lining of the cyst.

A tubular structure that was present in one of the sections was lined with cuboidal epithelium, and was about 0.75 mm. in its greatest diameter. One and one-half millimeters to the right of this in the section was a strip of stratified squamous epithelium 1.5 mm. by 0.1 mm., showing that this tubular structure lay not far beneath the skin. Slightly farther to the right and below in this same section were scattered, invading glandlike structures and part of the epithelial lining of the cyst. This tubular structure was almost an exact duplication of one of the tubular structures depicted by Mallory<sup>5a</sup> in his article describing remnants of medullary tube found in fetuses. I believe this to have been a persistent remnant of medullary tube, which communicated with the subdural space of the spinal canal. The subsequent history of the case supports this contention.

*Course in Hospital.*—At 3:30 p. m. on the day of operation, the patient complained of headache, and at 8:30 p. m. when the dressing was changed it was said to be saturated with serous discharge. On the next day, the patient complained of both frontal and occipital headache, which was described as terrible. Nausea, vomiting and free perspiration occurred. Dressings were again saturated with serous discharge. Headache became a daily feature, practically continuous, except when temporarily relieved by medication. The presence of watery discharge was noted whenever the dressing was changed. The temperature began to be elevated, at first to 99 F., then to 101 F. It was realized that cerebrospinal fluid was leaking from the incision. To help prevent excessive loss of spinal fluid, the foot of the bed was elevated.

The patient's condition gradually improved; the discharge of spinal fluid and the headache gradually became less. By the end of the second month after operation, there was little if any discharge of spinal fluid, and the patient usually made no complaint of headache. She began to sit up in a chair for a part of each day, and was dismissed from the hospital about the beginning of the third month after operation.

*Course After Leaving Hospital.*—During the fourth month after operation, enlarged glands were noted in the groin. It was considered that these were metastases.

During the fifth month after operation, the patient began to have high fever and complained of pain at the site of operation. On rectal examination, a bulging mass could be felt behind the rectum. There was a small opening through the old incision that had never entirely closed. Through this opening, a quantity of semi-necrotic tissue was curetted away, and the patient had relief while there was free drainage. Two or three weeks later radium was inserted. At this time "malignant granulations" were present about the wound. For about a month the patient felt better.

During the sixth month after operation, the patient began to cough, and about two weeks later there were signs of consolidation in the right lung. These pulmonary symptoms were interpreted as signs of metastases in the lung. About this time, also, the patient began to have recurrent vomiting.

About the end of the seventh month after operation, a mass was felt in the sigmoid region; the stools became very small and ribbon-like, and visible peristalsis was noted. The patient lost weight and died about eight months after operation. A necropsy was not obtained.

#### COMMENT

Middledorpf<sup>17</sup> was the first to attribute a congenital sacrococcygeal tumor to postanal intestine. His was the first reported sacrococcygeal

17. Middledorpf, K.: Virchows Arch. f. path. Anat. **101**:37, 1885.

tumor to contain intestine only. Those previously reported by Freyer<sup>4</sup> and others contained connective tissue, bone, cartilage or muscle in addition to intestine. The tumor in Middledorpf's case, that of a girl 1 year old, was successfully removed by Kraske. It consisted of small loops of intestine lying embedded in fat behind the rectum and adherent to the latter in an area about the size of a small finger nail. In the latter respect, it resembled the case that I have now reported. Bland-Sutton<sup>1b</sup> stated that he had considered postanal intestine the source of intestine-containing tumors in this region even before Middledorpf reported his case. Borst<sup>1a</sup> considered that tumors situated on the anterior surface of the sacrum and coccyx containing intestine-like structures were derived from postanal intestine, but he found no recorded case of carcinoma that might have arisen from this source. Carless<sup>18</sup> said that congenital adenoma of postanal intestine is innocent. Ewing<sup>1c</sup> agreed that a number of tumors in this region are derived from postanal intestine, but did not refer to the possibility of malignancy in this connection.

Hundling<sup>2</sup> reported two ventral tumors of the sacrum, which were carcinomatous. One was diagnosed as colloid carcinoma; the other as adenocarcinoma. The epithelium in the latter was cuboidal. In the reports of these two cases, no opinion is expressed as to their origin, but in the general discussion postanal intestine and remnants of neural tube ("ependymal cells") are referred to as possible sources.

Fletcher and Waring<sup>14</sup> reported an adenocarcinoma which they considered was derived from postanal intestine. This occurred in a boy, 2 years old, and was made up of a firm portion consisting of cysts lined with columnar epithelium, embedded in fibrous tissue, and a soft adenocarcinomatous portion. The tumor was removed, but death followed recurrence. At necropsy, tumor tissue was found enveloping without actually invading the rectum.<sup>19</sup> The rectum was narrowed at the pelvic brim, and the intestine above it was distended. There were metastases in the iliac and lumbar glands. The clinical history in the case that I have reported suggests a somewhat similar mode of extension.

Kraske<sup>6c</sup> observed two cases that he considered to be cases of carcinoma of postanal intestine, based on the postrectal position, relation to surrounding structures and microscopic appearance. Rostock<sup>6d</sup> quoted Galletly<sup>13</sup> as having reported another case, but examination of the original report does not substantiate Rostock's statement. Galletly con-

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18. Carless, A., in Rose and Carless: *Text-Book of Surgery*, Toronto, J. F. Hartz Company, 1924, p. 809.

19. Kraske<sup>6c</sup> stressed this noninvasion of the rectum in carcinoma of postanal intestine.

sidered his case to be one of tumor of the neurenteric canal, but made no suggestion that it was malignant. It is true that there were two recurrences of this tumor at intervals of years after removal, but this was apparently due to incomplete removal rather than to malignancy. Rostock also reported a case of his own, an adenocarcinoma, which, he concluded, was from postanal intestine. Beyond saying that it was "an infiltrating epithelial tumor of glandular structure," he gave no histologic details to establish his claim. Therefore, the type and origin of this adenocarcinoma remain in doubt. This was also the opinion of Dukes,<sup>20</sup> expressed in a review of Rostock's report.

It seems probable that the intestinal portions of some of the more complex congenital sacrococcygeal tumors have arisen from postanal intestine. This is the view of Nasse,<sup>21</sup> Borst,<sup>1a</sup> Bland-Sutton<sup>1b</sup> and Ewing.<sup>1c</sup> Stewart, Alter and Craig<sup>6b</sup> collected four cases of what they termed sacrococcygeal teratoma with malignant degeneration in childhood, and reported one case of their own. In only one of these cases did the evidence point strongly to an origin of the malignant tissue from postanal intestine, and there appears to have been no evidence to warrant classifying the tumor as a teratoma, since only epithelium and fibrous tissue were found in it. This was the case of Fletcher and Waring,<sup>14</sup> which has been discussed.

#### SUMMARY

It seems to me that the evidence in the case now reported is sufficient to justify the diagnosis of adenocarcinoma of postanal intestine. The criteria required by Peyron<sup>8</sup> in order to establish the diagnosis of postanal intestine cyst are present in this case, viz., columnar epithelium, goblet cells, presence in the lumina of material staining like mucus and absence of muscle or serous coat to the cyst. Peyron's opinion is based on many years of study of abnormalities of animals and of dissection of human embryos. Although there is evidence that the medullary tube was persistent below the exit of the sacral canal, yet this took no part in the formation of the tumor, the tumor being purely one of postanal intestine. There was no evidence of gliomatous tissue in this case. Neither was anything found to suggest a teratoid nature. Although many microscopic sections were made, no tissues were found other than those described. Only four other cases have been reported as carcinoma of the postanal intestine. The evidence in one of these cases is scant and unconvincing. In a few other cases, an origin of carcinoma from postanal intestine appears to have been suggested indirectly.

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20. Dukes, C. E.: *Cancer Rev.* 3:421, 1928.

21. Quoted by Borst<sup>1a</sup> and Ewing.<sup>1c</sup>

# BANDS AND RIDGES IN THE PULMONARY ARTERY

THEIR RELATION TO AYERZA'S DISEASE

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Cordlike structures within the vascular system constitute a very rare finding. Occasionally, they are found in the right auricle, forming a netlike structure. If present in the aorta and pulmonary arteries, they usually are located just above the area of the valves. In these locations they are, as a rule, regarded as congenital anomalies and explained on the basis of an abnormal developmental course of the septum within the truncus arteriosus. Bands in branches of the pulmonary artery, however, are, as far as could be determined, described only by Möller<sup>1</sup> and by Steinberg.<sup>2</sup> The results of a study of bands and ridges in the pulmonary arteries are now reported. A congenital anomaly as an underlying cause of these formations could be ruled out.

## LITERATURE

The literature on this subject is scant. Posselt<sup>3</sup> described a band in the pulmonary artery extending from the right cusp of the pulmonary valve to the intima of the pulmonary artery. In another case he mentioned a band in a segment of the pulmonary artery at a distance of about 1 cm. from the pulmonary valve. This band was 3 cm. long and had its origin and insertion in the intima of the artery. Its midportion crossed part of the lumen. Even though the bands were present in two old people, the author believed that they occurred on the basis of a congenital anomaly. Zahn<sup>4</sup> found a peculiar netlike structure in the left pulmonary artery of a 52 year old man who had died of pulmonary emboli. This net consisted of strings and thin membranes, which were attached to the intima, and which crossed parts of the lumen of the

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1. Möller, P.: Beitr. z. path. Anat. u. z. allg. Path. **71**:27, 1923.
2. Steinberg, U.: Beitr. z. path. Anat. u. z. allg. Path. **82**:307 and 443, 1929.
3. Posselt, A.: Ergebn. d. allg. Path. u. path. Anat. **13**:298, 1909.
4. Zahn, F. W.: Virchows Arch. f. Path. **115**:47, 1889.

pulmonary artery. Zahn did not believe that these structures were the result of organized thrombi, but ventured the opinion that they represented a congenital anomaly. Röhrle<sup>5</sup> described a cordlike structure in the aorta just above the aortic valve in a 2 weeks old child. He also mentioned the museum specimens of Nickiforoff and of Rosenberg, which revealed bands in a similar location. Chiari<sup>6</sup> and also Mönckeberg<sup>7</sup> described netlike structures in the right auricle. They were regarded as remnants of the valvula venosa dextra and of the septum spurium. Mönckeberg, however, remarked that in Rosenberg's specimen the band might represent excessive fenestration of the aortic valve. Poscharissky<sup>8</sup> described a band just above the pulmonary valve, and expressed the belief that the band originally was a part of the pulmonary valve, which had been torn away, probably because of excessive fenestration. It is interesting to note that Mönckeberg stated that such "anomalies" occasionally might be produced by a faulty opening of blood vessels. Artefacts therefore must be ruled out in considering the etiology of bands in vessels. Schober<sup>9</sup> also explained by excessive fenestration of the cusps a cordlike structure found just above the aortic valve in a 4 months old child. Lucksch<sup>10</sup> described a thin cord running across the lumen of the ascending aorta in a man aged 76. Arteriosclerotic lesions were absent. The cause of this formation was explained by an abnormal course of the septum in the truncus arteriosus. Möller<sup>1</sup> found bands in the pulmonary artery in four cases. Because of the fact that in three instances iron-containing pigment was found in the bands, he thought that the bands represented organized thrombi. A detailed histologic description, however, is missing. Steinberg<sup>2</sup> observed several bands connecting thickened areas of the intima of the pulmonary arteries. He noted in detail the changes of the pulmonary arteries histologically, but did not mention the finer structures of the bands. Verse<sup>11</sup> described bandlike structures in the splenic vein in a case of cavernous transformation of the periportal tissue with old thrombosis of the portal vein. These structures were regarded as newly formed blood vessels with marked intimal proliferation which had led to almost complete obliteration of their lumina.

5. Röhrle, F.: *Deutsche med. Wchnschr.* **22**:270, 1896.

6. Chiari, H.: *Beitr. z. path. Anat. u. z. allg. Path.* **22**:1, 1897.

7. Mönckeberg, J. G.: *Deutsche med. Wchnschr.* **33**:1243, 1907; *Die Missbildungen des Herzens*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, J. Springer, 1924.

8. Poscharissky, J. G.: *Beitr. z. path. Anat. u. z. allg. Path.* **35**:521, 1904.

9. Schober, F., in *Studien zur Pathologie der Entwicklung*, Jena, 1920, vol. 2, p. 527.

10. Lucksch, F.: *Centralbl. f. allg. Path. u. path. Anat.* **23**:626, 1912.

11. Verse, M.: *Beitr. z. path. Anat. u. z. allg. Path.* **48**:526, 1910.



The study here presented is based on examination of bands in the pulmonary artery in one case and of ridges in the pulmonary artery in another case. Both structures were encountered in the course of postmortem examinations.

#### DESCRIPTION OF CASES

CASE 1.—The clinical diagnosis in the case of a man, 60 years old, was: generalized arteriosclerosis, arterial hypertension and cardiac decompensation. There was no evidence of pulmonary arteriosclerosis, such as marked dyspnea or cyanosis. At postmortem examination, the heart weighed 850 Gm., was hypertrophic and dilated, and contained mural thrombi in the right auricle. There were diffuse



Fig. 1 (case 1) —Pulmonary artery opened. Note bands.

arteriosclerosis, coronary sclerosis with myocardial fibrosis, bilateral nephrosclerosis of the arteriolar variety and edema of both lower extremities. The lungs were distended and emphysematous. When the main branches of the pulmonary arteries were opened, a number of bands were noted on both sides. They were covered with intima and were yellowish and smooth. Some were flat and thick, others thin and delicate. The former, as a rule, were short, measured less than the diameter of the pulmonary artery, and crossed transversely a small portion of the lumen. The latter were much longer and crossed almost the entire lumen. The bands measured from 3 to 10 mm. in length. They arose from and had insertion in portions of thickened intima. They were not present in the common pulmonary artery, but were found in the main right and main left branch and also in the smaller distributions. On cross-section, the bands were light gray and showed a few darker red spots. In several instances emboli were found, caught apparently between the

intima of the pulmonary artery and the bands. There were many areas of intimal thickening in the pulmonary arteries, yellow areas of fatty degeneration, and some hyalinization. No atheromatous ulcers were noted.

Portions of the pulmonary artery with the bands were hardened in 10 per cent formaldehyde and embedded in paraffin. Serial sections were cut from one of the bands. Every third section was stained with hematoxylin-eosin, with a combination of iron-hematoxylin and orcein, and according to the van Gieson method. From some bands and portions of thickened intima of the pulmonary arteries, frozen sections were cut and stained for fat with sudan III.

Sections taken from the pulmonary artery close to the insertion of the band revealed marked intimal proliferation, with connective tissue formation and hyalini-

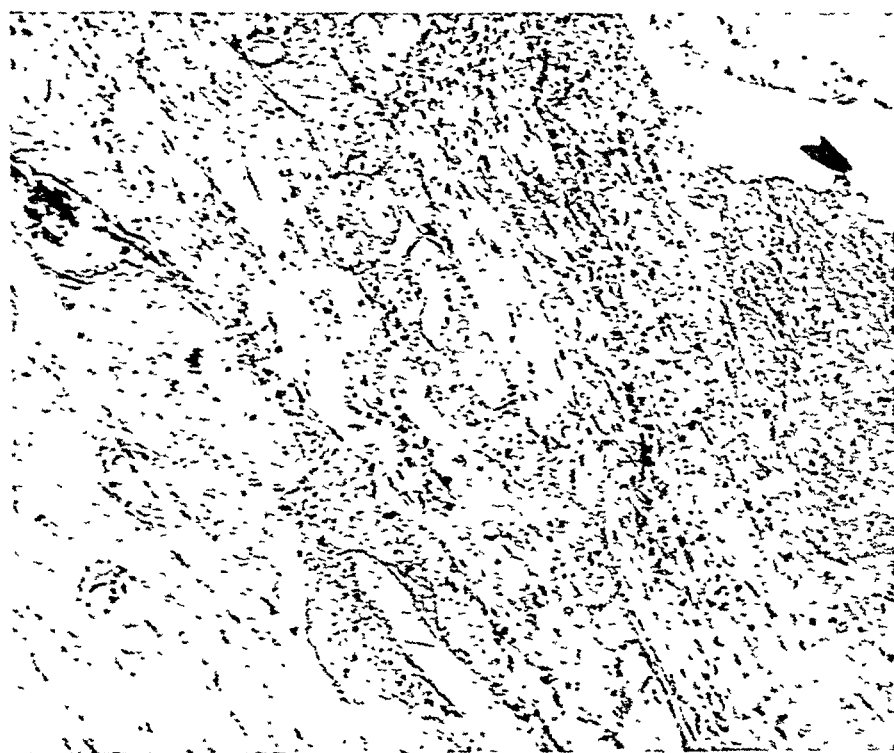


Fig. 2.—Structures resembling blood sinuses. Note fibrous cord lined by endothelial cells: iron-hematoxylin-eosin preparation:  $\times 100$ .

zation. Stained for fat, these portions revealed a moderate number of fat globules. Only a few spindle-shaped cells and, occasionally, lymphocytes were noted. There were small blood vessels in the intima. The media contained thick elastic fibers. No foci of lymphocytic infiltration could be demonstrated in the adventitia.

Sections taken from the points of insertion of the bands into the intima of the pulmonary artery showed much more connective tissue, which formed knoblike projections into the lumen of the artery. In some portions, the fibers seemed very loose and spread by an edema-like material. Several stellate-shaped cells with processes were found in this region. There were many small, newly formed blood vessels and a moderate number of lymphocytes and endothelial cells, some of which contained brown, granular pigment. In one area within the proliferated intima but close to the media, there was a large amount of fibrin, with many blood platelets,

red blood corpuscles, and clumps of dark brown, apparently hemosiderotic pigment. Lymphocytes and endothelial cells were found at the periphery of this region. A capsule of connective tissue separated this area from the surrounding intima and media. Serial sections revealed that these structures were present where the band was attached to the wall of the pulmonary artery, but that they did not extend into the neighboring intima. The media in this region revealed thick elastic and muscle fibers; but no cellular infiltrations were found. The adventitia showed no changes.

Sections taken from the bands themselves showed many connective tissue cells, loose fibers and small-sized blood vessels. There were many endothelial cells, some of which were filled with pigment granules, lymphocytes and, occasionally, poly-

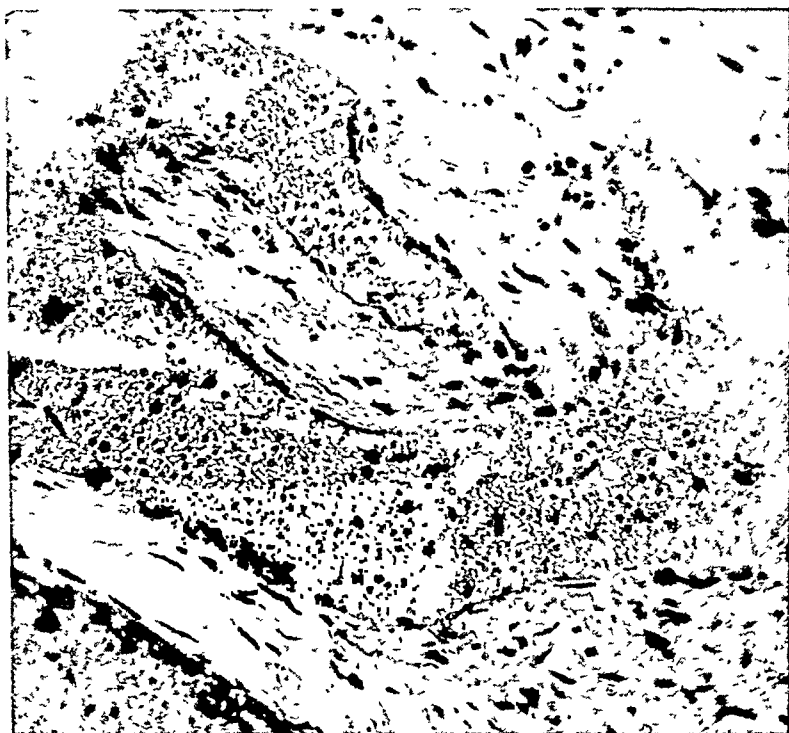


Fig. 3.—Field similar to that in figure 2; iron-hematoxylin-eosin preparation;  $\times 300$ .

morphonuclear leukocytes. Some portions of the band showed dense masses of pigment granules, strips of fibrin with many red blood corpuscles, masses of platelets, and more connective tissue than in the end-portions of the bands. Some sections revealed dense connective tissue fibers with hyalinization and only a few spindle-shaped cells. Many sections revealed large encapsulated areas with many red blood corpuscles. From the capsule, connective tissue fibers in the form of papillae or fibrous cores lined by endothelial cells extended into the hemorrhagic zone. In some portions, these fibrous cores had almost entirely replaced the hematoma, while in others large blood sinuses were still present, which were bound by connective tissue processes lined by endothelial cells. These formations were seen in many sections but were more pronounced in those taken from the midportions of the bands. The orcein stain showed thin elastic fibers throughout the bands.

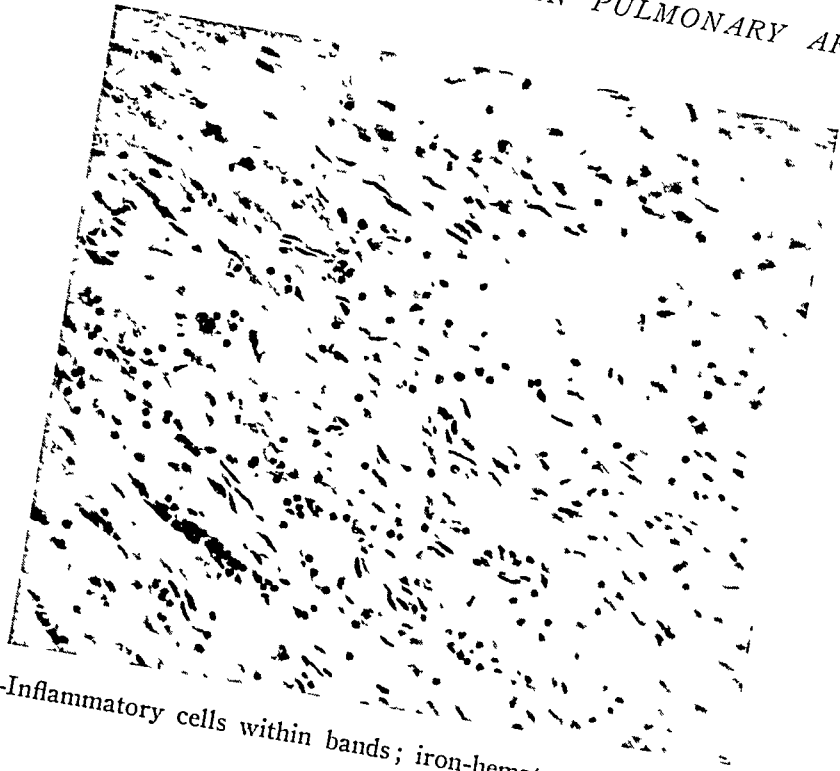


Fig. 4.—Inflammatory cells within bands; iron-hematoxylin-orcein preparation;  $\times 200$ .

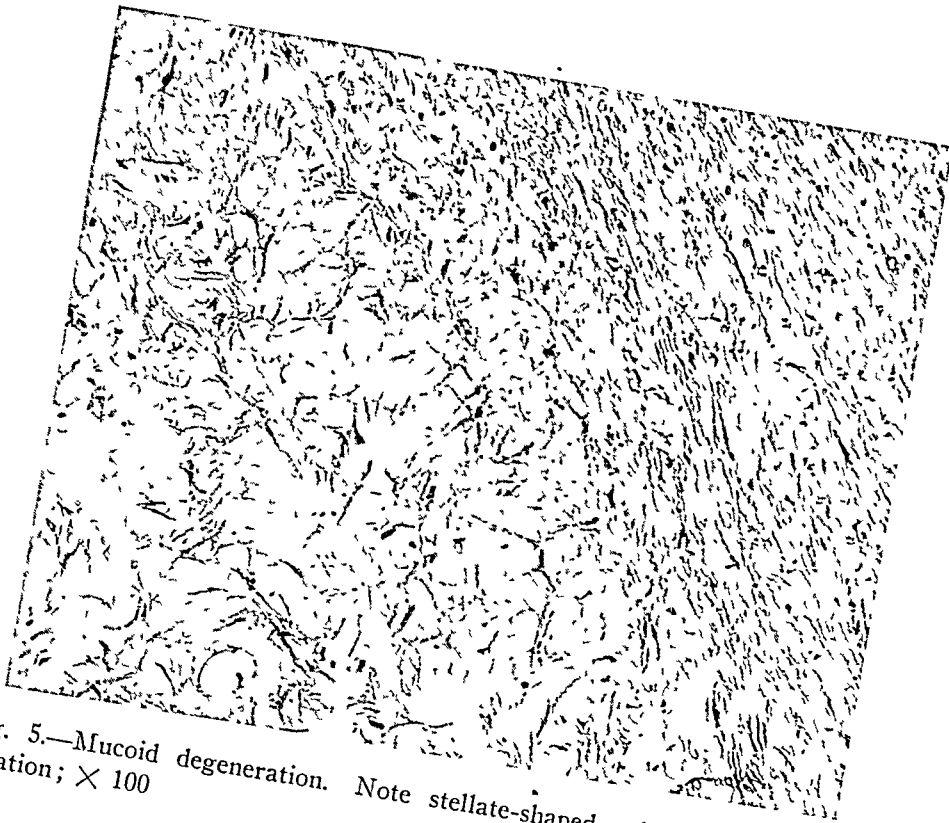


Fig. 5.—Mucoid degeneration. Note stellate-shaped cells; hematoxylin-eosin preparation;  $\times 100$



Fig. 6 (case 2).—Pulmonary artery opened. Arrow is directed toward one of ridges.



Fig. 7.—Arteriosclerosis of pulmonary artery; iron-hematoxylin-orcein preparation;  $\times 100$ .

To summarize—a patient with arterial hypertension and nephrosclerosis of the arteriolar variety, who died from cardiac decompensation, showed many bandlike structures in the pulmonary arteries, in addition to arteriosclerotic plaques. Histologically, the bands consisted of young and old connective tissue, with hyalinization. There were endothelial cells and lymphocytes present, also many red blood cells and pigmented areas. Newly formed blood vessels and blood sinuses were found throughout. The bands themselves were lined by intimal lining cells. In short, the bands represented organized and still organizing thrombi.

CASE 2.—The clinical diagnosis in the case of a 49 year old man was cardiac decompensation with diffuse arteriosclerosis. The patient did not show marked dyspnea or cyanosis, and there was no evidence, clinically, of pulmonary arteriosclerosis.

The autopsy revealed diffuse arteriosclerosis, nephrosclerosis of the arteriolar variety, and emphysema of the lungs. The heart was hypertrophic and dilated, weighing 650 Gm. The pulmonary artery appeared somewhat wider than normal. The common pulmonary artery and its branches showed many arteriosclerotic plaques. In some of the branches, several yellow ridgelike elevations of the intima were found, extending perpendicular to the long axis of the vessels. The ridges were sharp and well circumscribed, measuring up to 10 mm. in length and about 2 mm. in width. They were found between orifices of branches of the pulmonary arteries but were not present at the branching points. None of the ridges were undermined. There was no evidence of formation of bands.

On microscopic examination, these ridges showed a moderate connective tissue stroma with a few red blood cells dispersed throughout. There was some hyalinization. Small blood vessels were present, extending from the wall of the artery into the wall of the ridges. Lymphocytes and endothelial cells were found between the connective tissue fibers. Some of the endothelial cells contained pigment granules, but these were also seen free in the tissue. Some sections still showed old fibrin with blood platelets and a few polymorphonuclear leukocytes. The ridges were covered by intimal lining cells, forming a distinct continuation of the lining cells of the surrounding intima. The pulmonary artery in the region of the ridges showed much fibrosis and hyalinization. Occasionally, fat globules were noted in frozen sections. A few small-sized blood vessels were found in the intima, some of which were obliterated. The adventitia and media showed no changes.

To summarize—in a patient who died from cardiac decompensation associated with diffuse arteriosclerosis, nephrosclerosis of the arteriolar variety and emphysema of the lungs, ridges were found in the pulmonary artery. Histologic examination showed that these ridges were organized mural thrombi covered by intimal lining cells.

#### COMMENT

In the first case, bands were found crossing the lumina of branches of the pulmonary arteries. The sections revealed that these bands were organized thrombi covered by intimal lining cells. The pulmonary

arteries were the seat of moderate pulmonary arteriosclerosis. It is likely that the thrombi originally were formed on roughened areas of the intima because of atherosclerotic changes in the pulmonary arteries. This question, however, will be discussed subsequently. It is possible that organization of the thrombi had started more actively in only one or two places, while the remainder of the thrombi were detached from the wall, either because of shrinkage of the thrombus or because of the systolic pressure of the blood stream. This pressure must also have molded the thrombi in such a fashion as to form the bands. There is no evidence that a congenital anomaly might have been the underlying cause for the formation of the bands in this case. The fact alone that the bands were found in branches of the pulmonary artery makes such an origin improbable.

The ridges found in the second case resembled, at the first glance, so-called spurs found in the intima of the pulmonary artery close to the orifices of the smaller branches. But while such spurs histologically show the structures of the normal wall, with possibly some plain intimal thickening, the ridges here reported proved to be organized mural thrombi covered with intimal lining cells.

The literature contains only a few references to bandlike structures in blood vessels. As was mentioned before, Möller<sup>1</sup> found bands in the pulmonary artery which he interpreted as results of organized emboli. He did not mention arteriosclerotic lesions in the pulmonary arteries in his cases. Because of a possible relation of thrombi and bands, respectively, to pulmonary arteriosclerosis, the literature on pulmonary arteriosclerosis was searched for a description of bands. Only Steinberg,<sup>2</sup> in one of his two cases of primary pulmonary arteriosclerosis, mentioned structures apparently similar to those described in this paper. As was mentioned before, however, he did not give a histologic description of the bands. Grafe<sup>12</sup> and also Hart<sup>13</sup> mentioned the finding of thrombi in pulmonary arteriosclerosis. Ljungdahl<sup>14</sup> stated that in most cases of arteriosclerosis of the systemic circulation there is also revealed some pulmonary arteriosclerosis. Both of the patients whose lesions are described in this paper were over 60 years of age and had marked diffuse arteriosclerosis.

A number of cases of marked pulmonary arteriosclerosis are reported in the literature as Ayerza's disease.

The question arises whether or not the cases described here may be classified as cases of Ayerza's disease with pulmonary arteriosclerosis,

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12. Grafe: München. med. Wchnschr. **70**:1352, 1923.

13. Hart, C.: Berl. klin. Wchnschr. **53**:304, 1916.

14. Ljungdahl, M.: Untersuchungen über die Arteriosklerose des kleinen Kreislaufes, Munich, J. F. Bergmann, 1915.

mural thrombi and subsequent formation of bands and ridges. Cheney<sup>15</sup> stated that cough, dyspnea, cyanosis and somnolence are the characteristic clinical symptoms. Edema is usually a late occurrence. The patients appear intensely cyanotic and present other signs and symptoms of a severe cardiac decompensation. The red blood corpuscle count is usually over 5,000,000 and may reach 10,000,000. In six of eight cases in which the result of the Wassermann test was recorded, according to Cheney, the reaction was positive. Both of the patients concerned in the present study were over 60 years old. Both showed hypertensive heart failure clinically, and generalized arteriosclerosis, nephrosclerosis of the arteriolar variety, emphysema and hypertrophic and dilated hearts at autopsy. The Wassermann reaction in both cases was negative. The red blood count was 4,220,000 and 4,600,000, respectively. If, as Cheney stated, Ayerza's disease signifies a primary pulmonary arteriosclerosis resulting mainly in hypertrophy of the right ventricle, the two cases reported here are not examples of Ayerza's disease. The cardiac failure may easily be explained by the nephrosclerosis and the diffuse arteriosclerosis. The right ventricular hypertrophy in both cases was the result of the emphysema. The sclerosis of the pulmonary artery, especially the bands and ridges, might have been an additional factor in causing hypertrophy of the right side of the heart. The cause of the pulmonary arteriosclerosis in cases of Ayerza's disease, is, according to Cheney, usually or probably always syphilitic. In his case, Cheney described a striking round cell infiltration of the vasa vasorum of the adventitia and loss of the elastic fibers in the media of the pulmonary arteries. In the cases described here there was no evidence of syphilis clinically, serologically or histologically.

Structures somewhat similar to the bands and ridges reported here are encountered frequently in the endocardium of the left ventricle in cases of incompetence of the aortic valve, in the form of plain endocardial thickenings, bands or endocardial pockets. Only rarely, they are found in the auricles. As was shown in a previous paper,<sup>16</sup> endocardial thickenings and endocardial pockets are the result of either mechanical irritation or mural endocarditis. In other words, they might signify end-stages of healed thrombi. The pressure of the blood stream secondarily often undermines the thickened areas, producing bands or, sometimes, pockets. The bands in the pulmonary arteries of the first case showed a similar origin. There was a primary thrombosis which had undergone organization. These bands, similar to those in the endocardium, were formed secondarily, apparently by the force of the systolic pressure in the pulmonary circulation.

15. Cheney, G.: *Am. J. M. Sc.* **174**:34, 1927.

16. Saphir, O.: *Am. J. Path.* **6**:733, 1930.



In the foregoing paragraphs, the term thrombus was used to designate the primary lesion in the pulmonary artery. Whether the primary lesion was really a thrombus rather than an embolus is, of course, difficult to decide, especially because of the fact that mural thrombi were found in the auricles in the first case and also recent emboli in branches of the pulmonary artery. In both cases, arteriosclerotic changes were present with hyalinization and fatty changes, but no atheromatous ulcers. It does not seem justifiable to assume that such a hypothetic ulcer, which had healed, might have formed the basis of the mural thrombus. I also should like to mention Ljungdahl's statement that atheromatous ulcers in pulmonary arteriosclerosis are extremely rare. It seems much more likely that intimal thickenings and the roughened intimal surfaces alone suffice to cause the formation of thrombi. Mönckeberg<sup>7</sup> also stated that in one of his two reported cases of pulmonary arteriosclerosis, mural thrombi were found on circumscribed thickened areas of the intima, but not on an atheromatous ulcer. The emboli in the pulmonary artery in the first case were recent. It is interesting to note that they had lodged between the bands and the intima, where they were firmly implanted but not organized.

#### SUMMARY

Multiple bandlike structures in the pulmonary arteries are described in one case, and ridgelike formations in another. These structures are the result of organized mural thrombi. It is believed that the systolic pressure in the pulmonary artery transformed the thrombi into bands. A congenital anomaly could be ruled out as the underlying cause of these structures. Pulmonary arteriosclerosis was the primary cause of the mural thrombi. There was no evidence of Ayerza's disease.

# RELATION OF FIBRO-ADENOMA AND CHRONIC MASTITIS TO SEXUAL CYCLE CHANGES IN THE BREAST

HELEN INGLEBY, M.B., M.R.C.P.

PHILADELPHIA

In 1907, Hitschmann and Adler<sup>1</sup> described the endometrium in the different stages of the menstrual cycle, thus throwing an entirely new light on lesions of the uterus. It is known that these changes are correlated with the development and the regression of the corpus luteum. In 1922, Rosenberg<sup>2</sup> cut sections of breast and uterus in cases coming to autopsy and found a similar sexual cycle in the breast. Polano<sup>3</sup> and Sebening<sup>4</sup> confirmed these findings in surgical cases, but denied that postmenstrual regression is always complete.

Before considering pathologic changes it is necessary to examine the normal sexual cycle in the breast. The normal resting breast (about the middle ten days of the cycle) consists of fibrous tissue and ducts. In older women and in obese young women fat is present also. The ducts are lined by two layers of cells (often more than two are seen). The cells of the inner layer are cuboid or columnar. The nuclei are small and stain deeply. In good preparations, the dark staining may be seen to be due to a very fine, close chromatin meshwork. A nucleolus is also present. The protoplasm is scanty, so that the nuclei almost touch each other. The cell outlines are ill-defined, but toward the lumen of the ducts the epithelium presents a regular even line. The basal cells are smaller and tend to be flattened. As the time of the menstrual period approaches, the cells become larger, they divide and new ductules are formed branching out from the old ducts like twigs on a tree. The epithelial border around the lumen becomes somewhat jagged and irregular, a change similar to that seen in the glands of the premenstrual endometrium. Meanwhile the periductal fibrous tissue softens, undergoing myxomatous and hyaline degeneration so as to allow for the expansion of the ducts. The perilobular fibrous tissue is pushed to one side. In this way lobules that do not

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1. Hitschmann, F., and Adler, L.: *Wien. med. Wchnschr.* **57**:1297, 1907.
2. Rosenberg: *Frankfurt. Ztschr. f. Path.* **27**:466, 1922; *Virchows Arch. f. path. Anat.* **262**:298, 1926.
3. Polano, O.: *Ztschr. f. Geburtsh. u. Gynäk.* **87**:363, 1924.
4. Sebening, W.: *Arch. f. klin. Chir.* **134**:464, 1925.

exist in the normal resting breast are formed by the terminal portion of a duct and its branches. The epithelial cells swell, the protoplasm is often vacuolated, and the nuclei are rounder and paler, the chromatin meshwork being spread apart by a colorless substance. Similar changes are seen in the cells of the basal layer. Secretion takes place into the ducts, which become more or less distended, the amount of proliferation as well as of secretion differing considerably in different persons. In many women, the breasts become swollen and tender at this stage. About a day or so before the onset of the menstrual flow, involution begins. The epithelium degenerates. The cells of the basement layer especially become extremely vacuolated, and cleavage takes place along this line. Toward the end of the period, the degenerated epithelium is shed into the lumen of the ducts, much as the superficial layers of the endometrium are cast off during menstruation. Under the microscope, the lobules at the postmenstrual stage have a curiously jumbled appearance. The architecture of the ductules is lost, and one sees irregularly staining, degenerated epithelial cells, often varying in size and shape, intermingled with round cells and proliferating fibrous tissue. In the late postmenstrual phase, the degenerated epithelial cells are absorbed; for a short time, clefts may be seen in the tissue where the ducts have been, but these disappear, and the breast returns to the resting condition.

The amount of new growth taking place in the breast at each sexual cycle is astonishing. Premenstrual proliferation is much more rapid than carcinomatous growth. A point to be borne in mind is that the connective tissue of the breast proliferates and degenerates in inverse ratio to the epithelium; and although it would appear that the perilobular tissue is subject to compression by the growing ducts and to expansion when the pressure is removed, yet as regards periductal tissue there seem to be true growth and degeneration. A factor that must be allowed for in any discussion of the structure of the breast is that, even in normal breasts, different parts show differences in rate of proliferation and regression, so that at any given moment the lobules are not all exactly alike. McFarland<sup>5</sup> emphasized this with regard to involution after lactation, and the same is true of postmenstrual involution. Under pathologic conditions, as will be seen later, these differences become more marked.

Transplants of mammary tissue show the same cyclic changes,<sup>6</sup> thus proving their origin in hormonal influences. The substances regulating this growth seem to be secreted by the graafian follicle and the corpus luteum of the ovary under the influence of hormone or hormones from

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5. McFarland, J. J.: Surg., Gynec. & Obst. **45**:729, 1927.

6. Parkes, A. S.: Internal Secretions of the Ovary, New York, Longmans, Green & Co., 1929.

the anterior lobe of the pituitary gland. Experiments indicate that the corpus luteum is responsible for hyperplasia of the parenchymatous tissue of the breast. The following is a summary of recent work in this direction: <sup>7</sup>

1. Many workers have induced hypertrophy of the mammae in normal and in ovariectomized animals, in normal and in castrated male guinea-pigs, in young male dogs and in castrated monkeys by injecting estrin. This hypertrophy, no matter how prolonged the injections of estrin, has never been equal to the development of the breasts in pseudopregnancy or pregnancy, and is probably comparable to the hypertrophy observed at each estrual cycle. The conclusion of most observers is that estrin cannot cause complete development of the mammary gland.

2. The development of the mammary gland in pseudopregnancy and pregnancy seems to be definitely associated with the corpus luteum, for the following reasons:

(a) In animals with a short corpus luteum stage in the ovarian cycle there is hardly any change in the breast outside of the estrual proliferation.

(b) In animals with a long corpus luteum stage and in those having pseudopregnancies there is considerable development of the breasts at that time, followed even by secretion of milk.

(c) In those animals in which the corpus luteum stage is normally short and in which this has been prolonged artificially, the growth of the mammae can be brought up to equal the condition in pregnancy.

(d) "None of the changes characteristic of the luteal phase are found after ovariectomy or removal of corpora lutea." <sup>7f</sup>

With these facts in mind, the clinical and pathologic aspects of the matter may be considered. It was held until recently that inadequate development of the graafian follicle caused chronic hyperplasia of the endometrium because, although the stimulus for growth might not be so powerful as when a normal corpus luteum was formed, the stimulus for regression did not occur, and the proper shedding of the endometrium was interfered with. Each time that a follicle attempted to ripen the endometrium would grow, but since the follicle failed to mature, the cycle was not completed. If only a small portion of the endometrium was thus affected, the result would be the so-called mucous polyp. Applying this theory to the breast, Moszkowicz <sup>8</sup> con-

7. (a) Engle, E. T.: *Proc. Soc. Exper. Biol. & Med.* **25**:715, 1928. (b) Evans, H. M., and Simpson, M. E.: *ibid.* **26**:595, 597 and 598, 1928-1929. (c) Nelson, W. O., and Pfiffner, J. J.: *ibid.* **28**:1, 1930. (d) Zondek, B.: *Klin. Wchnschr.* **9**:241, 1930; *abstr.*, *J. A. M. A.* **94**:1272, 1930. (e) Parkes, A. S.: *Internal Secretions of the Ovary*, New York, Longmans, Green & Co., 1929. (f) *Brit. M. J.* **2**:1635, 1931.

8. Moszkowicz, L.: *Arch. f. klin. Chir.* **144**:138, 1927.

tended that so-called chronic mastitis was a generalized irregular hyperplasia, and fibro-adenoma, a localized hyperplasia of mammary tissues, and that both had their origin in ovarian dysfunction. However, this theory cannot be accepted in its entirety, for, as is now known, menstruation occurs in the absence of ovulation and formation of corpus luteum and can take place from interval endometrium. It is therefore not set in action by the withdrawal of the corpus luteum hormone. Allen<sup>9</sup> showed that menstruation could be brought on in ovariectomized monkeys after a series of injections of estrin. Recently Hartman<sup>10</sup> proved that estrin had no effect in the absence of the pituitary gland. If this is true at least two hormones are at work in bringing about sexual cyclic changes. One from the corpus luteum (itself a product of stimulation by the pituitary gland) is concerned in hyperplasia of endometrium and breast; one from the pituitary gland (activated by estrin from the ovary) causes involution.

Moszkowicz's theory has therefore to be widened to include pituitary dysfunction also. He himself did not offer proof of his hypothesis, and much experimental work has yet to be done before it can be definitely established. Still the theory forms a starting point for further investigation.

The easiest method of approach is to inquire into the origin of the growth called by various names, such as "fibro-adenoma," "adenofibroma," "periductal fibroma" and its varieties. If it is true that all these growths are the result of a local aberration of the menstrual cycle, the names lose their importance. They are only descriptive adjectives and serve merely to indicate different appearances in what is essentially a single pathologic entity. In the same way, a surgeon may speak of a carcinoma as "scirrhus," by which he means that there is a particular reaction of the patient's tissues, not any essential difference in the carcinoma as such. This example is not quite on all fours with fibro-adenoma, because in the latter the fibrous tissue is a part of the lesion; in carcinoma, it arises as a reaction of the host, but the analogy will serve. If fibro-adenoma is the result of a local irregularity of the cyclic changes in the breast, it must be produced by proliferation (which may be normal or may be excessive or irregular) followed by incomplete regression or by no regression. If this excess of growth over regression takes place at each cycle, it is easy to see that the result will be a tumor. As the tumor grows, it pushes aside the mammary tissue, thus forming a capsule for itself. The ultimate appearance of the growth of course depends on which of the tissues have

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9. Quoted by Parkes.<sup>6</sup>

10. Hartman, C. G.; Firor, W. M., and Geiling, E. M. K.: *Am. J. Physiol.* **95**:662, 1930.

been most concerned in the proliferation. Just as in a mucous polyp the proportion of glands to stroma is not constant, so in fibro-adenoma the proportion of epithelium to fibrous tissue varies. Moreover, the variation is much greater than in a uterine polyp, because in the breast fibrous proliferation and regression are a necessary part of the sexual cycle. This great variation in fibrous as well as in epithelial growth is the reason why so many names have been given to what is after all one pathologic entity. Suppose, for instance, that epithelial hyperplasia takes place during the premenstrual phase, but that no regression occurs. With each period, the affected lobules undergo further proliferation. The result is the so-called adenoma. If the cells come to the stage of secretion and no absorption occurs, the obvious result will be cysts of the acinar type. It is hardly necessary to postulate a blocked duct, for if there is no mechanism for collapsing the lobule, the cysts tend to remain in situ. When one remembers the comparative rarity of discharge from the nipple as a menstrual phenomenon, it appears that passage of menstrual secretion and shed cells into larger ducts does not take place on any great scale. Suppose, on the other hand, that the chief fault is an overgrowth of the fibrous tissue. The ducts try to proliferate in the premenstrual phase but they are pressed on and pulled out by the fibrous tissue, and the result is the ordinary intracanalicular type of fibro-adenoma.

Theoretically, therefore, the following elements are open to variation in fibro-adenoma (and this applies equally to the generalized lesions of "chronic mastitis" or mazoplasia): the amount of epithelial proliferation and regression and of lobule formation, the quantity of secretion poured into the ducts, and the growth and regression of the pericanalicular fibrous tissue. The possible combinations and permutations are too numerous to mention, but they more than account for the variations observed. The point now is to offer proofs of the correctness of this hypothesis.

If a fibro-adenoma is merely the result of local disturbance in the regular growth and involution of mammary tissue, it should be possible to trace in it some sort of sexual cycle, albeit an abnormal one. With this object, a number of cases have been investigated. For most of these I am indebted to my surgical colleagues, who were good enough to allow me to examine their patients and obtain the histories, and also to collect the tissues for examination immediately after operation. For the purpose of this study a full and reliable menstrual history with the date of onset of the last period was essential. This information is unfortunately missing in most hospital records, and, as so often happens in collecting cases, when a satisfactory history was recorded, the pathologic sections were incomplete or missing. For this reason, the

number of cases that could be usefully studied is small. However, through the kindness of the authorities of the Woman's Hospital and of the Lankenau Hospital, in both of which the records are exceptionally complete, it has been possible to bring the number of fibro-adenomas studied up to twenty-two. Whenever possible, a section from mammary tissue was taken as well as from the tumor. The material was fixed in Bouin's solution of picric acid and formaldehyde. Formaldehyde fixation, which is commonly used in routine work, is not satisfactory from the point of view of cytology, although this material shows general changes well enough.

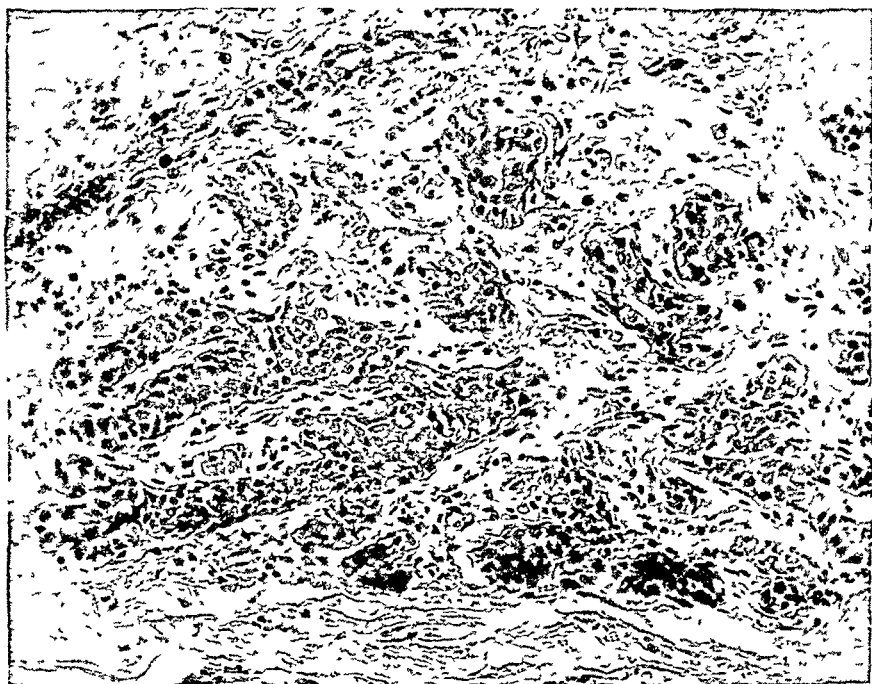


Fig. 1 (M. C., aged 35).—Breast six days before the onset of menstruation; part of a lobule showing branching of the ducts, premenstrual type of epithelium and periductal connective tissue;  $\times 200$ .

#### CYCLIC CHANGES IN FIBRO-ADENOMA

*Premenstrual* (Figs. 1 and 2).—M. C., aged 35, married, had had a mass in the left breast eight years, which had steadily increased in size. She menstruated regularly every twenty-eight days. The operation took place six days before a menstrual period was expected. The tumor, measuring 4 by 3 cm., was smooth and encapsulated. Microscopically, it consisted of ducts resembling those of the breast and more or less arranged in lobules. The lobules lay much closer together than in the normal breast, but they resembled those of the premenstrual phase. Some of the ductules were slightly dilated. Some were branched. The epithelium was premenstrual, i. e., the cells were large and pale, and the epithelial outline was irregular. This feature was most marked at the periphery of the tumor. The tissue between the ductules consisted of loose, edematous fibrous tissue. The breast

showed marked premenstrual proliferation, the lobules being exceptionally developed as regards both epithelial proliferation and loosening of the periductal tissue.

In this case, three characters of the premenstrual phase were present in the tumor—lobule formation, epithelial changes and loosening of the periductal fibrous tissue—although, as would be expected, none were as well developed as in the surrounding breast. Seven other patients ranging in age from 18 to 37 had fibro-adenomas removed during the premenstrual phase. In every case, the tumor showed definite premenstrual characters.

*Menstrual* (Figs. 3 and 4).—W. M. C., aged 23, single, noticed a growth following a blow received on the right breast when she was 13. The breast had



Fig. 2.—Fibro-adenoma six days before the onset of menstruation, from the same breast as the section pictured in figure 1. It shows lobule formation, branching of the ducts, premenstrual type of epithelium (although proliferation is not as marked as in the breast) and loose connective tissue;  $\times 200$ .

been tender at menstrual periods ever since, also when she had a cold and during attacks of tonsillitis. The growth remained unchanged, except that it enlarged slightly at the time of the menses.

The patient menstruated first at the age of 13; the menses were irregular at first, but at the time of examination were regular. The tumor was removed on the second day of a menstrual period. Grossly, it was a tough, white, lobulated mass, 3.5 by 1.5 cm. With it was a small piece of breast.

Microscopically, the breast was typical of the menstrual phase. Well formed lobules were present. The epithelium was swollen and irregular, and the cells of the basal layer were much vacuolated as though they were about to disintegrate with shedding of the whole epithelium as the result. The periductal connective



tissue was still edematous, but showed commencing proliferation, as is usual after menstruation. The tumor consisted of ducts and lobules resembling those of the breast, except that they were more closely packed together. The lobules were especially well marked toward the periphery of the tumor. The interlobular tissue was dense; the periductal tissue resembled that of the breast. The similarity of mammary and tumor epithelium was striking; in the tumor, the menstrual character was only slightly less developed than in the breast.

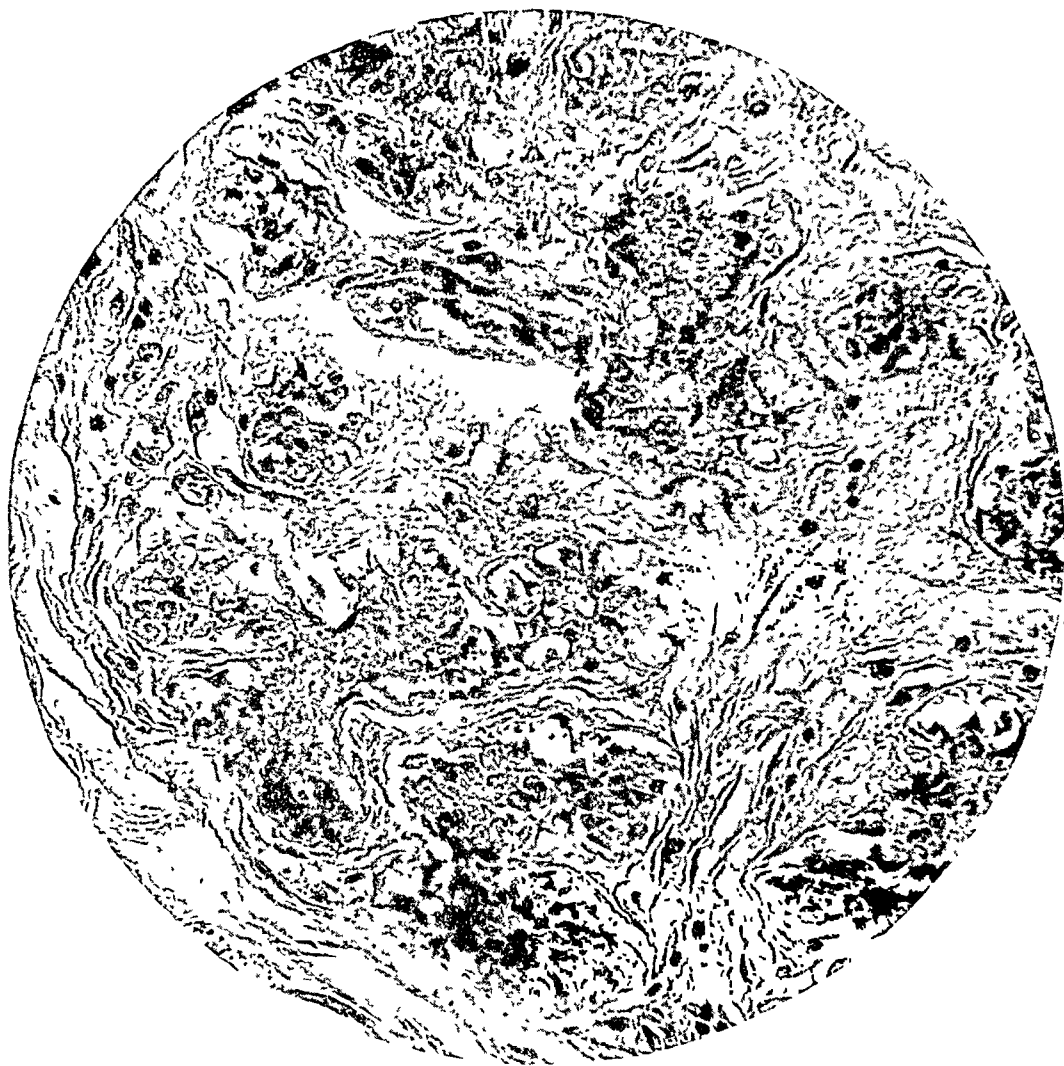


Fig. 3 (W. M. C., aged 23).—Breast on the second day of a menstrual period; high power magnification to show the normal vacuolation of the cells of the basement layer.

Two other tumors were removed during menstruation at a slightly later stage. They showed desquamation of epithelium in both tumor and breast.

*Postmenstrual* (Fig. 5).—S. N., 27, had had a tumor in the left breast for six months. She had been married for eleven months, with no pregnancy. The tumor was removed nine days after the commencement of a menstrual period. It was a lobulated, encapsulated mass, 3.5 by 2.5 cm. No mammary tissue was taken.

Microscopically, there were numbers of ducts embedded in fibrous tissue. Many of the ducts were considerably dilated. Some contained secretion. The epithelium was desquamated into the ducts in many places. Where it was present, the cells were small and regular (typical resting epithelium). In many places, a jumble of desquamated epithelial cells was found in the midst of connective tissue. The appearance was exactly like that seen in the postmenstrual phase in a normal breast, and undoubtedly represented lobules in process of involution. The connec-

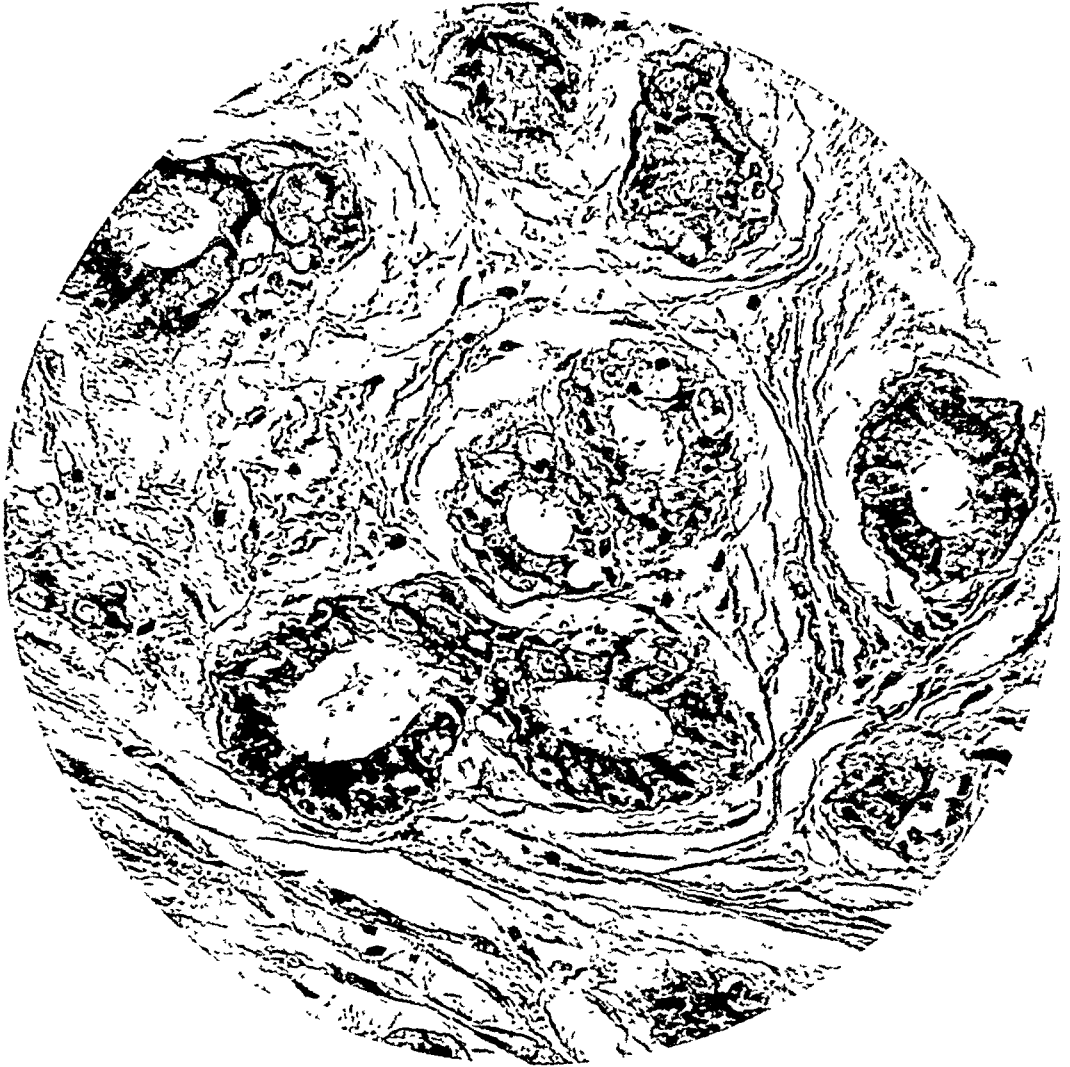


Fig. 4.—Fibro-adenoma on second day of menstruation, from the same breast as the specimen shown in figure 3. Vacuolation of the cells of the basement layer is marked, although not so advanced as in the breast. In both the periductal tissue is loose.

tive tissue was homogeneous and fairly dense as in the late postmenstrual or in the interval phase.

In this tumor, some lobules underwent involution, but not all. Those that did not regress remained as groups of dilated ducts, the lobule formation being gradually lost, owing, no doubt, partly to pressure of the surrounding fibrous tissue and partly to pressure of the cystic ducts themselves.

In another case of a tumor removed seven days after the onset of a menstrual period, postmenstrual regression had only just begun, but breast and tumor showed the same character.

*Interval* (Figs. 6 and 7).—S. F., 22, single, noticed a tumor eight months previous to operation. The breasts were painful before menstrual periods, but the tumor did not alter. The menses were regular; the rhythm,  $\frac{28}{5}$ . The onset had been at the age of 12. The tumor was removed fourteen days after the commencement of a menstrual period.

Microscopically, the tumor consisted of fibrous tissue in which were embedded elongated ducts. Nowhere in the tumor was there any indication of lobule formation. The epithelium lining the ducts was regular, the nuclei stained deeply, and

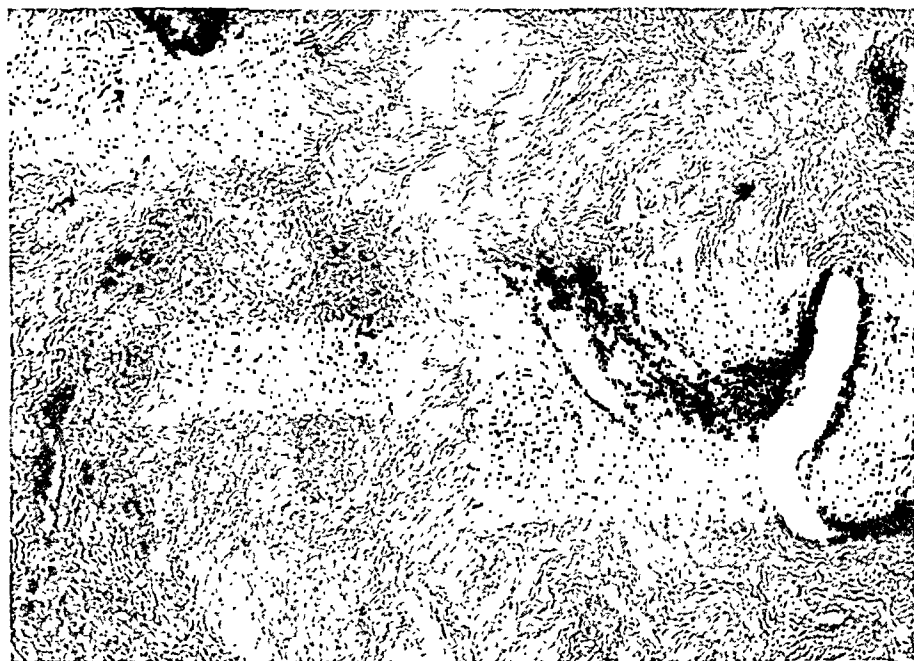


Fig. 5 (S. N., aged 27).—Fibro-adenoma removed nine days after the onset of menstruation. It shows well marked postmenstrual involution in some parts of the tumor and dilated ducts in others. Fairly dense connective tissue is shown;  $\times 50$ .

there was no vacuolation of the protoplasm. In the mammary tissue, the lobules varied a little. Most were typical of the interval phase; a few showed early premenstrual budding. Overgrowth of fibrous tissue was the most conspicuous feature of this tumor, possibly preventing complete regression of the ducts.

Three other cases in which the tumors were removed in the interval phase showed typical interval epithelium with no proliferation of ductules.

*Amenorrhic*.—One tumor, in a girl of 16, was removed during a period of amenorrhea. Microscopically, there was nothing characteristic of any special phase of the sexual cycle. There was irregular proliferation of the epithelium, but the cells were small. The connective tissue was loose, but lobules were not present.

*Postmenopause* (Fig. 8).—In E. A., aged 52, the menopause occurred ten years previous to operation. The lump in the breast had been noticed some weeks before operation.

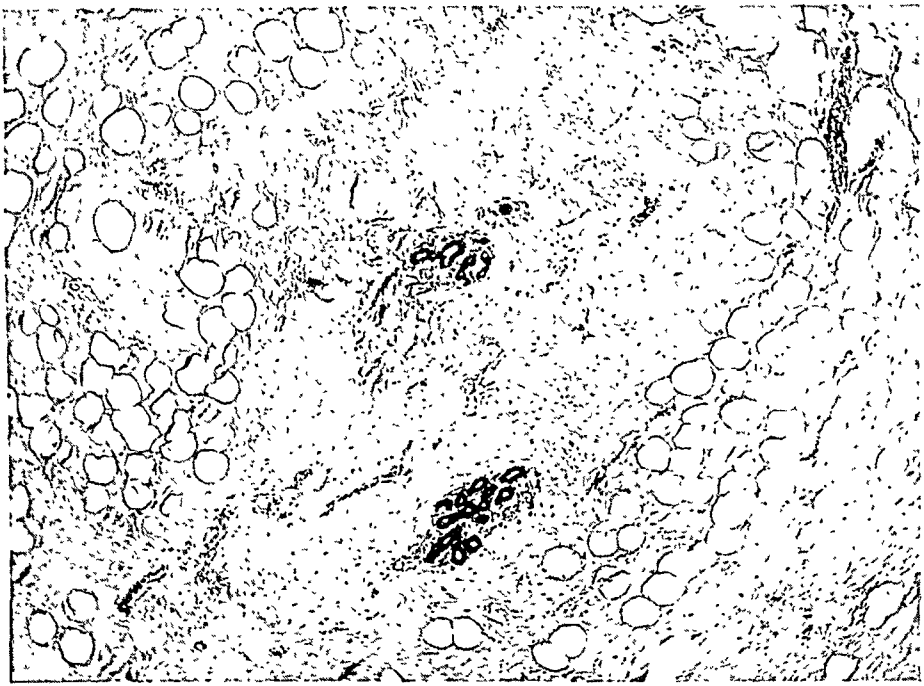


Fig. 6 (S. F., aged 22).—Breast fourteen days after the onset of menstruation—the interval stage—showing small groups of ducts with deeply staining, regular epithelium. Traces of former lobules that have undergone involution can be made out in the connective tissue.

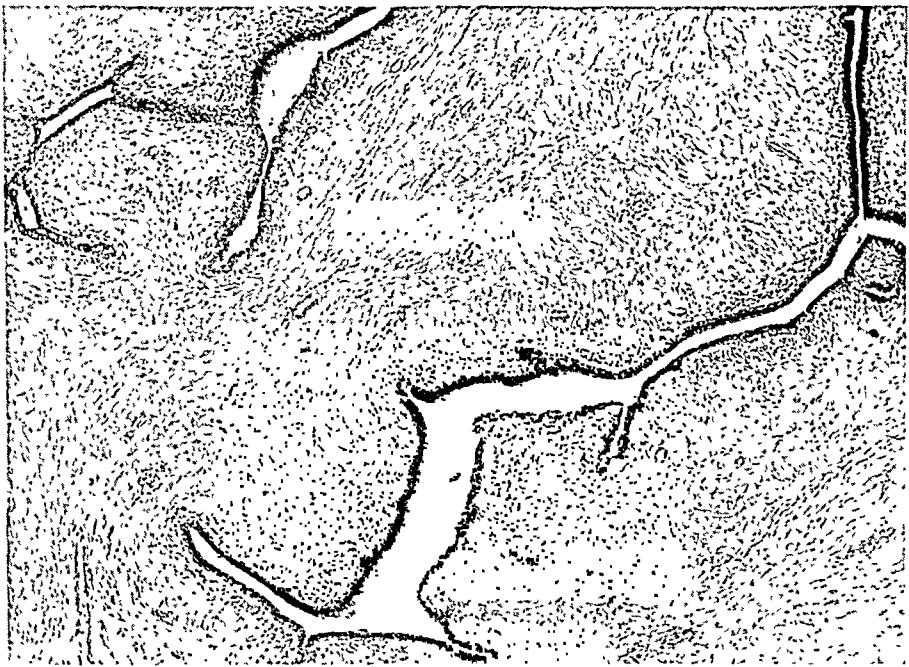


Fig. 7.—Fibro-adenoma fourteen days after menstruation from the same breast as the section shown in figure 6. There is no lobule formation. The epithelial cells are small. The fibrous tissue is deeply staining and uniformly dense; it appears to be rather more dense around the ducts.

Microscopically, the breast showed the ordinary senile changes. It consisted of fibrous tissue and fat, in which ductules with inactive epithelium could be seen here and there. The tumor consisted mainly of dense fibrous tissue. In some places, however, there were ducts that had been distorted by the growth of the fibrous tissue (so-called intracanalicular type of tumor). The epithelial cells were small and showed no sign of proliferation; some had been shed, leaving only a basement membrane. This tumor seems to have been caused purely by overgrowth of fibrous tissue. It showed nothing indicating any kind of sexual cyclic change.

The nineteen cases just described were uncomplicated in the sense that as far as was known there was no disturbance of mammary tissue

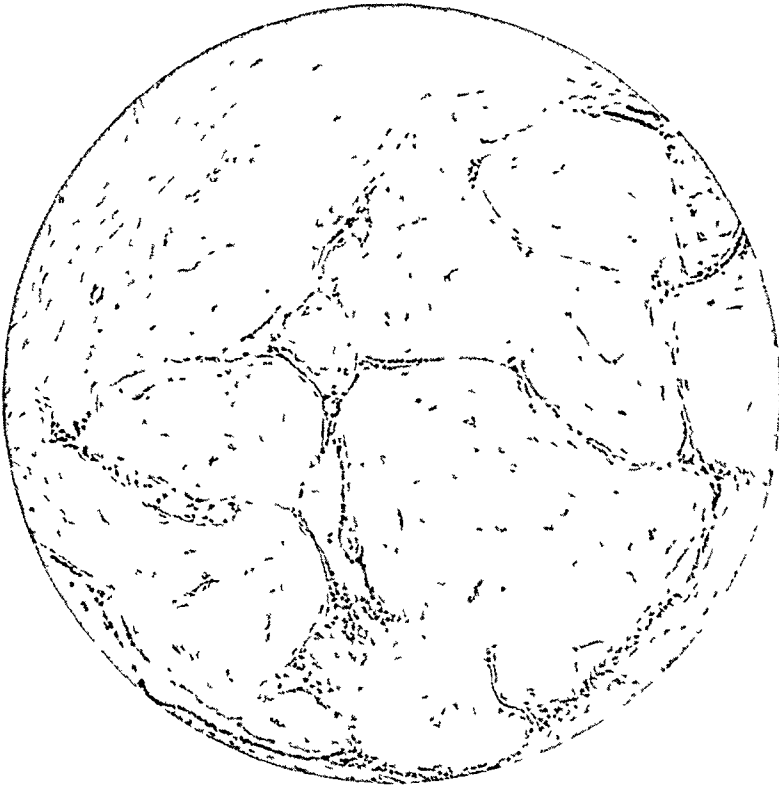


Fig. 8 (E. A., aged 52).—Fibro-adenoma ten years after the menopause, showing dense fibrous tissue, distorted ducts and degenerate epithelium.

other than the fibro-adenoma. In seventeen cases there was a regular or almost regular menstrual cycle. In the two in which tumors were removed during a period of amenorrhea and after the menopause, the growths showed no definite changes belonging to the sexual cycle. In the other tumors, the stage of the cycle could always be recognized, sometimes easily, sometimes only after careful study. The recognition of the cycle in tumors seemed difficult at first, but with practice it has been found possible to date tumors fairly accurately without other information than the microscopic section of the growth. It is not to be expected that all the changes that occur in the normal breast during

the sexual cycle will be found in tumors. If this happened, there would be no tumor. New lobule formation, for example, was found to some extent in all of the tumors removed during the premenstrual stage. It was generally seen near the edge of the growth, probably because the tissue was able to expand in this direction. The central parts did not show it nearly so often. Regression of lobules was seldom complete, and sometimes did not appear to have taken place. In the interval tumors, no definite lobular formation was present. The elongated, branched ducts so often seen are doubtless the distorted remains of lobules. One is enabled to judge between newly formed and old lobules because the latter are almost sure to be distorted by pressure of the surrounding tissues and by distention from their own secretion. The periductal tissue may agree with the stage of the sexual cycle, but is likely to be abnormal. In ten of fourteen cases it was definitely abnormal. Once it was excessively hyaline. Three times hyaline degeneration accompanied an excessively dense tissue. Once it was edematous and loose during the interval phase. In the remaining five cases, the fibrous tissue was dense and overgrown, i. e., excessive even for the interval phase. Probably the most constant criterion of the menstrual date is the appearance of the epithelial cells. In all these cases it was characteristic at least over the greater part of the tumor. As with lobule formation, the change tended to be best defined in the peripheral parts of the growth. In general, it may be said that while the indications of cyclic changes are clear, they are never exactly the same as in the normal breast. Usually the development is not perfect and lags behind that of the breast. Often it is excessive in certain directions (epithelial or fibrous tissue proliferation). The periphery of the growth is usually more nearly like the normal breast than the center. Often normal mammary lobules are caught up in the capsule, indicating that they may in turn come under the influence of aberrant stimuli and eventually form part of the tumor. However, the fact that new lobule formation takes place at the periphery of the growth suggests that it is proliferation in response to cyclic stimuli that is chiefly responsible for its increase in bulk.

Clinical confirmation of this view is found in the number of cases in which the tumor was first noticed just before a menstrual period, and in which an increase in size (sometimes followed by a decrease after the period) was noted at this time. Of seven patients of whom the question was specifically asked, two gave a history of the lumps being noticed just before a menstrual period and three others of increase in size just before or at the time of periods.

If proliferation occurs in a fibro-adenoma during the premenstrual phase, one might expect a much greater increase during pregnancy and

lactation. McFarland<sup>11</sup> reported such cases and described lactation in one tumor. A description of a case of lactation hyperplasia in a fibroadenoma follows:

M. F., aged 18, married, complained of a large tumor in the left breast. At 6 years of age she had fallen on the left breast. It was painful for some time, but she never noticed lumps until she began to menstruate at the age of 13. At this time, the left breast became somewhat painful and nodular, but did not increase in size. This continued for some time, but subsequently subsided and gave no trouble. The menstrual periods were regular. She was delivered of her first child ten months before coming to the hospital. Following this, she had a painful swelling in the left breast and was told she had a "caked breast." The swelling gradually increased and was painful at times. She did not nurse her child except for the first few days. On examination, a mass the size of a small grapefruit was found in the outer half of the breast. The tumor was firm and somewhat tender. The veins over the breast were markedly dilated. At operation the tumor was found to occupy the greater part of the breast. The breast was therefore removed along with the growth.

The tumor was a smooth encapsulated mass about 10 cm. in diameter. The cut surface was honeycombed with small cysts. These gave a somewhat spongy feel to the whole mass.

Microscopically, the tumor showed numerous very large, branched, distended ducts (fig. 9). The epithelial cells lining them had an unusual appearance, for in most places a sort of tuft projected from their protoplasm. This was evidently a cell secretion, for the tufts varied in shape, and sometimes the material formed a rounded mass that hung by a thread from its parent cell. The nuclei of the cells were fairly large and clear staining. The cells of the basal layer were swollen and vacuolated. Near the large dilated ducts, groups of smaller ones showing the characters of a lactating breast might be found. Sometimes the large ducts were embedded in fat, but more often they were surrounded by peculiar cellular areas, which frequently filled the spaces between them. In these areas, irregular, vacuolated cells lay in clumps or were scattered through the tissue. They had the same characters as the epithelial cells, but their nuclei were extremely irregular and vacuolated, and their protoplasm was reduced to shreds, giving them a stellate appearance resembling that seen in myxomatous tissue. Sometimes remains of ducts could be made out.

These areas with their absence of any regular architecture were comparable to the postmenstrual phase of the sexual cycle and recalled strongly the appearance seen in involution following lactation. The disorganization could not have been due merely to pressure from the dilated ducts, because in some places, as has been said, groups of regular ductules were present. In this tumor, lactation involution seems to have progressed up to a certain point, but not to have been satisfactorily completed. It would seem that the stimulus toward lactation had not ceased to act.

The breast was reduced to a shell around the tumor and was therefore much compressed. In spite of the compression it showed well marked involution of the type that follows lactation. There were large lobules, the outer part of which consisted of young fibrous tissue and the center of branching ducts, many of which were dilated and filled with epithelial cells in various stages of degeneration. Sometimes ductules were replaced by a jumble of cells; the cells resembled those of the tumor but were smaller.

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11. Deaver, J., and McFarland, J.: *The Breast*, Philadelphia, P. Blakiston's Son & Co., 1916, p. 447, and illustrations, p. 448.

Dr. Otto Saphir of Chicago sent me particulars of a case on which he and Dr. Irving F. Stein are about to publish a report—a case of pregnancy hyperplasia in a fibro-adenoma. In a woman, aged 21, who had been pregnant for two months, a tumor of the left breast developed, which was removed immediately. The photomicrograph (fig. 10) shows proliferation of ducts and acini which, although irregular in places, bear considerable resemblance to those found in normal lobules

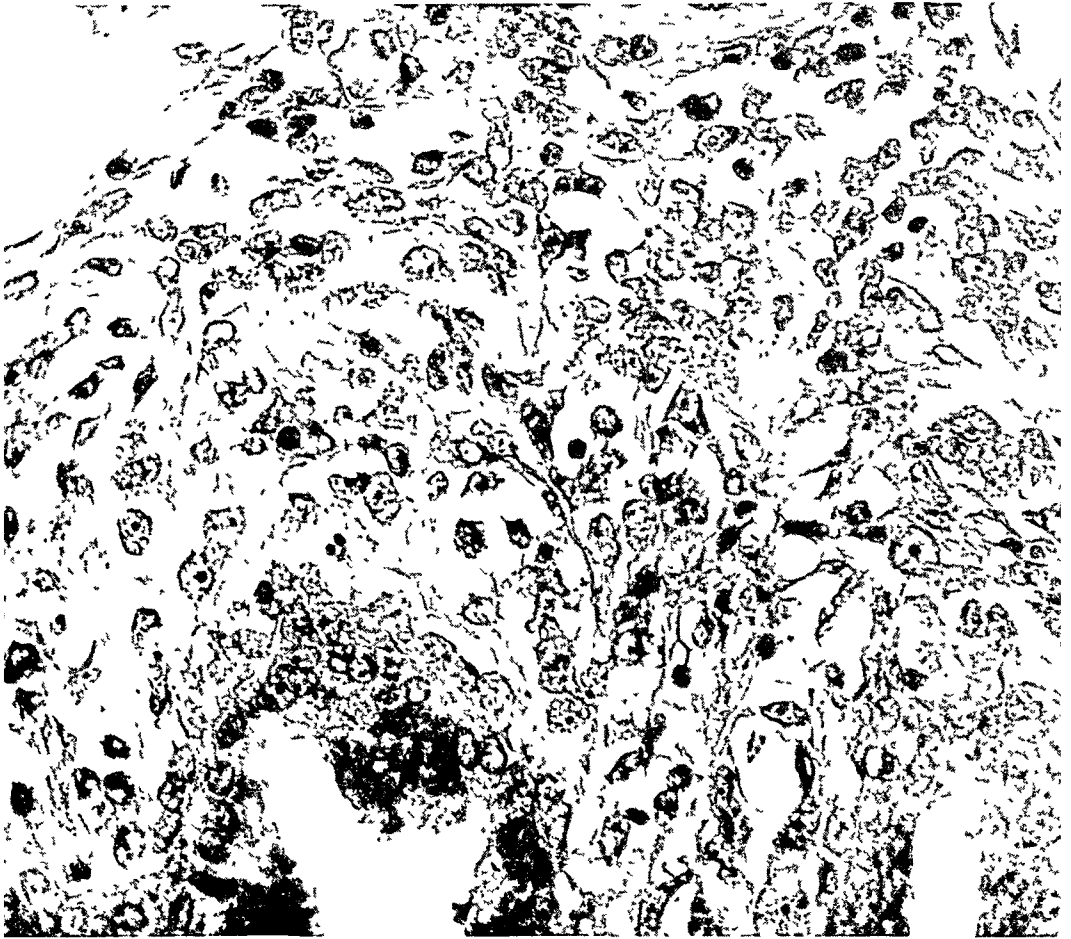


Fig. 9 (M. F., aged 18).—Fibro-adenoma of breast ten months after parturition; high power magnification to show lactation involution.

during pregnancy. The interesting point is that five pictures from different parts of the growth show absolutely no indication of involution, which was such a prominent feature in the lactation tumor described in the preceding paragraphs.

From a study of the cases described in this paper, one may fairly conclude that *the changes in a fibro-adenoma parallel those of the breast*. Nevertheless, it may be objected that this does not prove that the tumor first arose owing to a local aberration in the sexual cycle. There may



have been some other cause, and the sexual changes might be secondary. In reply one may say that of course there must have been an exciting cause, as perhaps trauma, which provoked the irregularity in one part of the breast rather than in another, but it seems extremely probable that the mechanism of formation of the tumor is as suggested. McFarland<sup>12</sup> in 1918 put forward the theory that "the tumor is primarily a lobule of breast tissue aroused to growth through unknown stimuli."

An argument in favor of fibro-adenoma being nothing more than the result of local dysfunction in a lobule is the difficulty often found

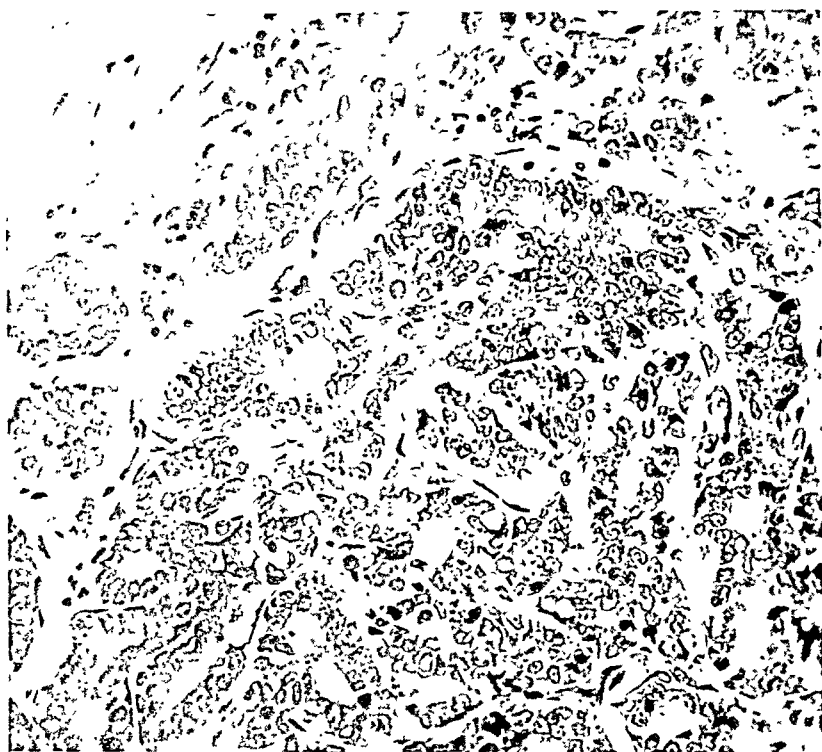


Fig. 10.—Dr. Saphir's case of a fibro-adenoma removed during pregnancy. It shows pregnancy hyperplasia.

in deciding from microscopic sections whether one is dealing with a tumor or not. In one case, after it had been decided that the section was that of a resting breast in which postmenstrual involution had not been complete, a gross description was found in which it was stated that the tumor was completely encapsulated and had been shelled out without the removal of any mammary tissue. McFarland<sup>5</sup> in his analysis of some three hundred cases was faced by the same difficulty; in one hundred and forty-seven cases called instances of "tumor" by surgeons and pathologists, he found only normal mammary tissue; in thirty-seven

12. McFarland, F.: *Arch. Surg.* 5:1, 1922.

cases, he found it impossible to decide whether a tumor was present or not. The same problem arose in this small series. Unless a gross description is available, it may be impossible to distinguish between a fibro-adenoma and one or other form of so-called chronic mastitis, which it is preferable to call mazoplasia after Cheate,<sup>13</sup> or even between fibro-adenoma and normal breast. The only distinction that would enable one to decide in these cases would be the presence or the absence of a capsule. Of course, some tumors have characters that do not show themselves in a generalized mazoplasia, such as the extreme pulling out of ducts by fibrous tissue, but a lesser degree of the same thing is often seen, and it would seem likely that special characters are the result as much of compression by the surrounding breast as of a particular kind of growth. Many of these tumors are semi-encapsulated showing complete continuity with normal breast on one side and sharp demarcation from mammary tissue on the other. It is easy to see how such a situation would arise from proliferation of a lobule or of a group of lobules.

Therefore since the transition from fibro-adenoma to mazoplasia is such a gradual one, and since the line between the normal and the abnormal in the breast is so hard to draw, it seems reasonable to postulate a gradual transition from the physiologic to the pathologic. In other words, aberrant physiologic stimuli are more likely to be the etiologic factor than an extraneous cause unconnected with the sexual cycle. The probability is enhanced if it is possible to show a close connection between hormonal disturbance and the pathologic condition in the breast.

#### MAMMARY CHANGES IN PATIENTS WITH IRREGULAR SEXUAL CYCLES

*Young Women.*—E. R., aged 17, single, had a small nodule in the right breast. Menstruation began when she was 17 years old. The periods were irregular, menstruation occurring about every two months. She menstruated forty-six days before operation, and she did not menstruate again until forty-six days after operation. She had three attacks of appendicitis before coming into the hospital. A chronically inflamed appendix was removed as well as a tumor of the breast. The tumor is said to have been "the size of a strawberry."

Microscopically, the mammary tissue showed every kind of irregularity. There were cystic ducts, some containing proliferated epithelium. Lobules were present, somewhat resembling those of the menstrual or postmenstrual phase, and in many places the dilated ducts showed a lobular grouping. The extreme, irregular epithelial proliferation would have earned the title of precancerous mastitis had the section been examined some years ago. The tumor showed groups of dilated ducts arranged more or less in lobular fashion in loose, edematous connective tissue. Two similar cases in girls aged 16 and 18, respectively, were studied.

13. Cheate, G. L.: Arch. Surg. 17:535, 1928.

Cheatle<sup>13</sup> in 1928 called attention to the resemblance between this condition and "chronic mastitis" and to the etiologic association between general active changes at puberty and the formation of fibro-adenoma.

*Woman of 33.*—L. C., aged 35, married, complained of a lump in the left breast, which had been present for six months, gradually increasing in size. She had been married eleven years and had had four children, also one miscarriage at two months one year before admission. The onset of menstruation was at the age of 13; the rhythm was  $\frac{30}{3-4}$ ; the menstrual periods were irregular, sometimes being as much as six weeks late. There was moderate dysmenorrhea. For the past two years, the flow had been very scanty and had lasted only one day; menstruation had been followed by epistaxis. Flushings and headaches took the place of periods. Such a condition occurred one week after operation for removal of the breast.

Microscopically, the breast showed premenstrual characters, but with considerable hyperplasia of lobules and ducts. There were numerous cysts filled with shed epithelium, also moderate hyperplasia of the lining membrane of the large cystic ducts. Around some of the ducts were groups of lymphocytes; probably they appeared in response to irritation by the degenerated contents of the cysts. No circumscribed tumor was found.

This patient reported again two and one-half years later. The periods were still scanty and slightly irregular, but much less so than when she was in the hospital. She complained of pain in the remaining right breast at times, the pain being worse before a period. On examination twenty days after the last period, one dilated duct could be felt in the breast.

In this case, it would seem that the condition of the breast improved with the improvement in regularity of the cycle, but the menstrual function was still abnormal, and the condition of the breast had not completely cleared up.

Cases of irregular proliferation of the breast in women around the age of 40 are so common that individual instances need not be recorded here. Many are associated with pelvic lesions. As is well known, menstrual irregularities tend to occur at puberty and at the menopause. In women nearing the menopause, even if menstruation is regular, various alterations in the body indicate an alteration in the balance of the hormones. Every surgeon is aware of how common lesions of the breast are at this time. It must be emphasized that, although irregularity of the menses is accompanied by irregularity of development and involution during the sexual cycle in the breast, it does not follow that the factor responsible for menstruation is the same as that governing the changes in the breast.

Stress has been laid on menstrual irregularity in mazoplasia because it is the most obvious and the most trustworthy evidence of hormonal alteration at one's disposal. It is the best index that one has that the cycle is disturbed. The converse, that because menstruation is regular the cycle is not disturbed, is obviously not true, as experimental work shows. Otherwise, the number of cases of mazoplasia in which

menstruation is regular would be amply sufficient to refute the argument put forward here. It would be exceedingly interesting to discover whether the endometrium is really normal in the latter cases, but there is not often the opportunity of examining specimens from the breast and endometrium of the same patient.

The next question is: Do cases of generalized mazoplasia occur in which ovarian dysfunction can be excluded? This is an extremely difficult question to answer. There is no doubt that certain causes will provoke an attack, but whether they will do so in the absence of any hormonal imbalance can probably be settled only by animal experiment. Since such attacks occur practically always in middle-aged women, it looks as though there must be an underlying hormonal factor. An important exciting cause is trauma. A history of trauma occurs frequently both in fibro-adenoma and in mazoplasia. In many cases, however, it is denied. Hammett,<sup>14</sup> demonstrated the part played by injury in releasing the growth-promoting SH radical. Injury undoubtedly increases any preexisting hyperplasia, and there seems no reason why it might not upset the normal growth and regression of the sexual cycle, although it is perhaps harder to see why it should continue its effects indefinitely. However, the continued effect might be attributed to mechanical disturbances from the proliferated fibrous tissue. Mechanical disturbances appear to be of considerable importance. Another exciting cause is the ingestion of certain substances, notably strawberries. Possibly certain persons might react by swelling of the breasts to any substance to which they were hypersensitive, but, as far as I know, no observations have been published on this point. Exposure to cold may be an exciting cause—it undoubtedly tends to increase mazoplasia when it is present, as also does the ingestion of alcohol, especially wines.

Inflammation, which has been regarded as such a potent factor in the past, has not been found an etiologic factor in the cases concerned here. Groups of round cells, it is true, are often present in the neighborhood of dilated ducts, but they can more easily be interpreted as an effect of irritation from the distended ducts and cell débris within them than as the cause of the lesion. The fact is that when inflammation occurs, it does not produce mazoplasia.

#### MASTITIS WITHOUT ABNORMAL CYCLIC CHANGES

E. W., aged 28, single, had suffered from soreness and enlarging and hardening of the right breast for six months. It was tender and seemed more prominent at

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14. Hammett, F. S. • *Protoplasma* 4:103, 1928; 7:297, 1929; *Proc. Am. Philos. Soc.* 68:151, 1929. Hammett, F. S., and Reimann, S. R.: *J. Exper. Med.* 50:445, 1929.

the menstrual periods. She had had otitis media. There was a discharge from the right ear while she was in the hospital. Menstruation was regular, with onset at 11 years and rhythm  $\frac{28}{6}$ . A diagnosis of chronic cystic mastitis was made, and the breast was removed fourteen days after a menstrual period.

Microscopically, the breast was in the interval phase. There was nothing suggestive of any disturbance of the sexual cycle. The chief abnormality was a great excess of young, branched fibrous tissue cells that compressed many of the ducts. Other ducts were surrounded by masses of lymphocytes, in far larger numbers than is seen in mazoplasia. This case could be truly labeled "chronic mastitis;" the lesion was obviously inflammatory.

Three other cases were studied. A girl of 19 had three abscesses in her breast in the course of a year. A sinus was excised on the first day of a menstrual period. In spite of severe inflammation, the adjacent tissue of the breast showed normal menstrual features.

A Negro woman of 40 had had a tuberculous infection of the breast for sixteen years. The organ was riddled with sinuses. It was excised just before a menstrual period was due. Where mammary tissue still remained, there was no disturbance in the development of the lobules, which were typically premenstrual.

In a woman of 39 who had irregular menstrual periods and also a chronic abscess, the mammary tissue at a distance from the abscess showed very irregular proliferation, while that within the zone of inflammation was more nearly normal.

The conclusion to be drawn from these cases would seem to be that inflammation alone does not affect the mammary cycle and is not a cause of mazoplasia. In view of the clinical connection between menstrual irregularity and mazoplasia it seemed worth while to see whether it was possible to produce changes similar to those of chronic mastitis in the breasts of animals by altering the estrual cycle.

In the rat, intercourse with a vasectomized male induces pseudo-pregnancy and changes the menstrual cycle from the normal four or five days to approximately ten days. As a preliminary experiment, ten female rats were placed with two vasectomized males. This was evidently too high a proportion of females, for in two of them the cycle did not change, and in the others there were periods of a return to the four day cycle. Four young rats showed regular increase in size of the lobules of the breast, the expected physiologic response to a prolonged cycle. Two older rats had a rather irregular epithelial hyperplasia, and two showed a well marked cystic condition of the breast with very irregular development of the lobules that were not cystic. In all the animals, pieces of mammary tissue removed before the experiment were normal. In order to be sure that the hyperplasia had nothing to do with trauma from previous operations, the experiment was repeated with nine new rats. In four, hyperplasia and cysts developed. It would be premature to draw conclusions from such small numbers. Investigations along these lines are being continued, and it is hoped to publish a full report later.

## SUMMARY

In twenty-one cases of fibro-adenoma of the breast, a comparison of tumor and breast from the same patient showed that in patients with a regular menstrual cycle the same type of cyclic change was found in the tumor as in the adjacent normal breast. It was found possible to diagnose the stage of the cycle from an examination of the tumor alone.

One tumor was removed during pregnancy and one after parturition. The former showed hyperplasia resembling that occurring during pregnancy; the latter had characters partly of lactating breast and partly of involution following lactation.

In four cases in which the menstrual periods were irregular, it was not possible to assign the tumor to any special phase of the cycle.

Hyperplasia with growth of new lobules occurs regularly in fibro-adenomas during the premenstrual phase of the sexual cycle. This is most easily seen at the edge of the tumor. Involution is often incomplete. Hyperplasia of periductal fibrous tissue may be in excess of the normal. It follows that a fibro-adenoma is the result of a local irregularity in growth and regression of the sexual cycle in the breast. What provokes the irregularity is not known.

Disturbance of the balance of hormones concerned with the sexual cycle may be considered the most important etiologic factor in mazoplasia. Cases are recorded to show the close association of so-called "chronic mastitis," or mazoplasia, with irregularity of the menstrual cycle, which is taken as an index of hormonal disturbance.

Inflammation is not an etiologic factor in mazoplasia. Three cases of severe chronic inflammation of the breast showed no irregularity in the sexual cyclic changes.

It was found possible to produce a cystic condition and irregular hyperplasia in the breasts of rats by upsetting the estrual cycle.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## VII. RESTORATION OF THE LIVER AFTER PARTIAL SURGICAL REMOVAL AND LIGATION OF THE BILE DUCT IN WHITE RATS

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AND

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Restoration of the liver to normal weight occurs rapidly after partial removal. The liver of the white rat recovered approximately 35 per cent of its preoperative weight the first day following removal,<sup>1</sup> and by the end of the third day it had more than doubled in weight. Factors incident to such a rapid rate of recovery of the preoperative weight of the liver are of great biologic interest, for no other organ, so far as we know, recovers its weight so rapidly following the removal of so large a portion of its parenchyma. Although the cytologic organization of the rapidly restored organ is not quite identical with that of a normal liver, yet there is no indication that any functional disturbance has been imposed. Complete restoration of the liver to its original weight, following removal of about 70 per cent of it, is ordinarily attained in from ten to fourteen days.

In a consideration of the factors that control or regulate this rapid restoration we were interested to know to what extent hepatic injury, such as that imposed by obstruction to biliary outflow, might modify normal recovery. Mann, Fishback, Gay and Green<sup>2</sup> (1931) reported that when biliary obstruction was induced in dogs by double ligation and section of the common bile duct before the liver was partially removed, such remarkable restoration did not occur.

### METHOD OF EXPERIMENT

Sixty rats, aged from 6 to 9 months, having a mean body weight of  $171.3 \pm 4.156$  Gm., were operated on. These rats were from the genetic strain used in the earlier studies on hepatic regeneration. With the aseptic method already

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From the Division of Experimental Surgery and Pathology.

1. Higgins, G. M., and Anderson, R. M.: Arch. Path. **12**:186, 1931.

2. Mann, F. C.; Fishback, F. C.; Gay, J. G., and Green, G. F.: Arch. Path. **12**:7899, 1931.

described, about 70 per cent of the liver was removed; at the same time, the bile duct was securely ligated but not sectioned. The rat does not have a gallbladder, and thus complications induced by the biliary vesicle in the presence of obstruction did not exist. All animals had free access to a 15 per cent dextrose solution immediately after the operation and were placed on the routine laboratory ration of food and water the following day. To secure data on the weights of the restored parenchyma and the ratios of weight of liver to weight of body following partial hepatectomy, five animals were killed by exsanguination at three days, one week, two weeks, three weeks and four weeks after the operation, and to secure early data with regard to changes in hepatic cells two rats were killed at eighteen, twenty-four, forty-eight and seventy-two hours after operation. Portions of livers were fixed in Zenker-formaldehyde solution and stained with hematoxylin and eosin, with eosin-azur II, and for iron.

*Mean Weights of Body and of Moist Liver Before Partial Hepatectomy and Ligation of Common Duct and at Intervals During Restoration*

Group	Animals	Lapse of Time After Operation Before Animals Were Killed	Mean Weights							
			Before Operation		At Time of Partial Hepatectomy		At Time of Death		During Restoration	
			Body. Gm.	Liver Gm.	Liver Re- moved, Gm.	Liver Remain ing, Gm.	Body, Gm.	Liver, Gm.	Hepatic Increment age of (Moist) Gm.	Percent- age of Weight of Liver
All animals operated on	60	.....	171.3 ± 4.156	6.21 ± 0.0769	4.36 ± 0.0720					
1.....	5	72 hours	146.8 ± 7.314	5.62 ± 0.2876	3.62 ± 0.0693	2.00 ± 0.2958	136.2 ± 6.289	4.72 ± 0.2960	2.72 ± 0.4184	0.0346
2.....	5	7 days	182.0 ± 11.82	6.48 ± 0.2876	4.46 ± 0.1753	2.02 ± 0.3368	148.2 ± 8.722	6.070 ± 0.3500	4.05 ± 0.4857	0.0409
3.....	5	14 days	188.4 ± 8.94	6.62 ± 0.2876	5.00 ± 0.3642	1.62 ± 0.4646	167.6 ± 7.910	6.950 ± 0.8490	5.33 ± 0.9677	0.0414
4.....	5	21 days	195.2 ± 13.05	6.78 ± 0.2876	4.45 ± 0.0570	2.33 ± 0.2932	184.8 ± 11.36	8.84 ± 0.4150	6.51 ± 0.5081	0.0478
5.....	5	28 days	225.0 ± 11.77	7.51 ± 0.2876	5.70 ± 0.4450	1.81 ± 0.5298	198.0 ± 10.05	6.81 ± 1.0800	5.00 ± 1.2030	0.0344

By means of the formula  $y = 0.024x + 2.1 \pm \frac{0.5752}{\sqrt{n-1}}$ , in which  $x$  is body weight and  $y$  is liver weight,<sup>1</sup> the preoperative weights of the livers of all experimental animals were estimated. Since the amount of liver removed surgically from each rat was known, the weight of the parenchyma that remained in the peritoneal space after the operation was easily estimated. The average gain in weight of the livers of the five rats killed at each of the designated intervals was computed. The onset and extent of jaundice were observed, and determinations of serum bilirubin were made at the time the animals were killed.

#### EXTENT AND RATE OF HEPATIC RESTORATION

It was clear from the data assembled during this study, and condensed as shown in table 1, that ligation of the bile duct at the time of partial hepatectomy induced considerable effect on the rate and the extent of the apparent restoration of the organ. The curve of the weights of the restored parenchyma in the presence of biliary obstruc-



tion may be readily contrasted with the curve of hepatic weights at corresponding intervals after partial hepatectomy alone (fig. 1 *a* and *b*).

At the end of the third day after operation, the actual increase in the weight of the liver for each 100 Gm. of preoperative body weight was 1.8 Gm. This is the exact figure computed at the end of the third day after partial removal and splenectomy,<sup>3</sup> and is slightly greater than that computed, after a corresponding interval, when partial hepatectomy alone was done.

At the end of the first week, the increase in weight of the liver was more marked in those animals in which biliary stasis had been induced. An average increase of 2.2 Gm. of parenchyma for each 100 Gm. of

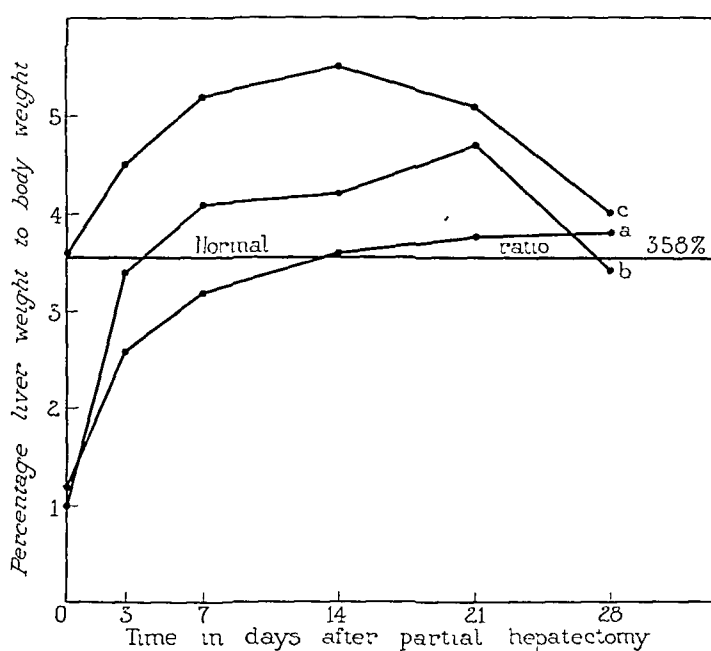


Fig. 1.—Percentage of weight of liver in relation to weight of body during hepatic restoration. The curves represent (*a*) restoration following partial hepatectomy; (*b*) restoration following partial hepatectomy and ligation of common bile duct; (*c*) liver weight per cent of body weight following ligation of common duct without removal of liver.

preoperative body weight was recorded for the rats with biliary obstruction, and 1.6 Gm. had been restored for a corresponding unit of body weight of animals subjected to partial hepatectomy only. The ratio of the weight of the liver to the weight of the body at the time of death was greater by approximately 1 per cent in the series with biliary obstruction.

At the end of the second week, the increase in the weight of the restored parenchyma in the control rats subjected to partial hepatectomy

3. Higgins, G. M., and Priestley, J. T.: Arch. Path. **13**:573, 1932.

only and that in the rats in which there was biliary obstruction were more nearly alike. On the basis of preoperative body weights, an average of 2.8 Gm. of hepatic parenchyma, for each 100 Gm., had developed in animals with obstruction, and only 2.4 Gm. in the control animals. At the end of the third week, however, the increase in weight of the restored parenchyma in animals with biliary obstruction was greater by 0.7 Gm., for each 100 Gm. of preoperative body weight, than in the control rats. Thus it appeared that, so far as the weights of the livers were concerned, biliary stasis had induced increased rates of hepatic restoration.

During the fourth week, the mortality was higher, and the animals that survived to the twenty-eighth day had patent bile ducts; the ligature had been absorbed and continuity with the duodenum was reestablished. Jaundice had disappeared, and determinations of the serum bilirubin were negative. The animals which survived for twenty-eight days, and which were killed at that time for data on the weights of the livers, were normal as far as bile drainage was concerned. It must be assumed, however, in the presence of jaundice and other gross manifestations, that these animals had passed through the period of temporary obstruction. When the data on weights of the livers assembled from these animals four weeks after operation were compared with those of the control rats, the extent of restoration throughout the period was much greater in the group without obstruction. This would seem to indicate that the increased weights recorded when obstruction was present were due not so much to actual restored parenchyma as to retention of bile constituents.

To test the effect of obstruction of the bile duct on the weight of the normal liver, forty rats were operated on. The bile ducts were ligated as before, but the liver was not removed. Total obstruction to the flow of bile induced an immediate increase in the weight of the liver (fig. 1 *c*). Strangely enough, the curve of the ratios of weight of liver to weight of body at the indicated intervals in this series was closely parallel for the first two weeks to that describing the ratios in the rats with biliary obstruction from which the livers had been removed. By the twenty-first day, however, the ratios were lower (fig. 1 *c*), and when biliary continuity was again established, essentially normal ratios were recorded.

Following such obstruction to biliary outflow by ligation, excessive dilatation of the bile duct proximal to the ligature occurred. These dilated ducts often appeared as large pouches, resembling gallbladders, on the inferior surface of the liver (fig. 2), and were often as large as 3 cm. in diameter, even distending the abdominal wall. Occasionally they ruptured, and bile peritonitis then ensued.

## JAUNDICE

Jaundice, as determined by its appearance in the sclera, the urine and the viscera, was never grossly visible until the second or third day. In some instances, however, determinations of serum bilirubin revealed, as early as eighteen hours after operation, bilirubinemia of 10.96 mg. for each 100 cc. of blood. Determinations were inconstant in that analysis of the serum varied greatly within any group of animals killed at any given time. The average of the serum bilirubin for all animals killed at intervals was 10.50 mg. at twenty-four hours, 16.05 mg. at forty-eight hours, 13.05 mg. at seventy-two hours, 17.21 mg.



Fig. 2.—Marked distention of bile duct proximal to ligature fourteen days after operation.

at seven days, 12.26 mg. at fourteen days, and 6.44 mg. at twenty-one days. Bilirubinemia was not observed in any animals killed on the twenty-eighth day. The bilirubinemia in animals in which only the duct was ligated did not vary to any extent from that which was determined for those with both hepatic removal and biliary obstruction. In the presence of total obstruction, the amounts of hepatic parenchyma present were without significant effect on the onset or on the extent of bilirubin in the blood.

## HEPATIC CYTOLOGIC CHANGES DURING RESTORATION

The cytologic changes that took place in the hepatic remnant during restoration were those of typical biliary cirrhosis with obstruction. Changes, which were induced as early as fifteen or eighteen hours, were progressive and gradually involved most of the lobule. Within a few

hours the canaliculi were distended with inspissated bile, and large globules of bile from ruptured canaliculi often distended the hepatic cells, pushing the nuclei to one side. Another early reaction was an increase in the number and the size of local histiocytes. Erythrocytes were commonly seen within them, and granules of hematoidin, with an unusually large amount of hemosiderin, occurred in the cytoplasm of these Kupffer cells. Obstruction to the flow of bile had induced retention within the cells of the pigments normally elaborated. During the second and third day, the cytoplasm of the hepatic cells was alveolar, filled with innumerable small globules of bile. Prussian blue stains

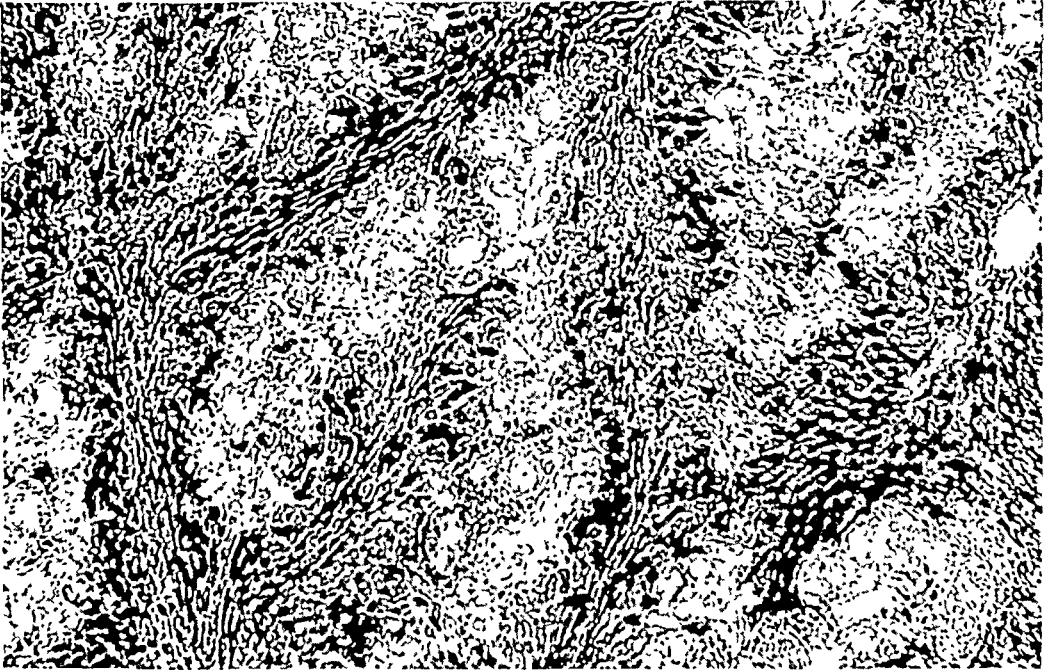


Fig. 3.—Biliary cirrhosis in liver of white rat twenty-one days after ligation of bile duct;  $\times 90$ .

revealed considerable iron within hepatic cells around these yellow-tinged bile deposits.

Besides the necrotic changes that occurred in the lobule, and the increase in local histiocytes, there was portal infiltration by lymphocytes and polymorphonuclear leukocytes. Marked distention of the extrahepatic bile duct proximal to the ligature occurred, but the intrahepatic ducts were also enlarged. Extensive pericholangitis developed around the proliferated newly formed bile ducts and ultimately formed wide zones of fibrous tissue around the reduced areas of functioning parenchyma (fig. 3). A study of the sections gave no evidence, however, that true hepatic restoration had occurred. The rapid increase in the size of the hepatic remnant following partial removal in any animal is probably associated with hypertrophy of the

hepatic cell and karyokinesis. Certainly by far the greatest increment is attained by an increase in the size of the preexisting cells, for cell division, although frequent during the second and third day, is not sufficient to produce so rapid an increase in weight. In the presence of biliary obstruction, these two cell reactions did not occur to any extent. Occasional mitotic figures were seen, and certainly the cellular hypertrophy which did occur was not comparable to that which ensues on partial removal, but more likely is due to factors associated with pressure induced by the intracellular retention of large quantities of inspissated bile.

The large ratios of weight of liver to weight of body that were recorded during the first, second and third weeks of this experiment are thus explained not as true restoration, such as ensues on partial removal alone, but as a result of actual distention of the lobules of the liver with constituents of bile and erythrocytes.

#### COMMENT AND SUMMARY

These experimental results, although differing somewhat from those of Mann, Fishback, Gay and Green,<sup>2</sup> rather definitely support their conclusion that restoration of the liver following partial removal does not take place extensively in the presence of biliary obstruction. They induced obstruction by ligation of the common bile duct in dogs at about thirty days before partial hepatectomy, whereas we combined removal with ligation in a single operation. In their study, restoration was induced long after the effects of obstruction had been imposed; in our study, the factors of restoration were operative coincidentally with the effects of obstruction to biliary outflow.

As far as size and weight of the liver are concerned, the results of partial hepatectomy in the presence of obstruction are not dissimilar to those of true restoration. Ligation of the duct induced in dogs, as it did in white rats, a greenish-brown organ, greatly distended and engorged. This engorgement with inspissated bile explains, we believe, the increased ratios of weight of liver to weight of body that were recorded and plotted in curve *c* of figure 1. Accordingly, from the weight of the organ alone one may not predict the extent of acutal restoration following removal that occurs in the presence of total obstruction. When hepatectomy was performed in the presence of biliary obstruction, a certain amount of hepatic restoration no doubt occurred before the distention, induced by the obstruction, interfered. The occasional mitotic figures noted on the second day, indicated that at least some restoration had taken place. Mitotic division was not observed in hepatic cells during the later periods of obstruction when large globules of bile had so distorted the cell as to dislodge the nucleus to an eccentric position.

The rapid rate of restoration that occurs in normal rats during the first two or three days following partial removal seems to be largely correlated with the volume of blood traversing the liver. In fact, observations now in progress in this laboratory lead us rather definitely to conclude that the entire principle of restoration following removal is definitely correlated with the amount of blood passing through the remaining portion of parenchyma after removal. Stephenson<sup>4</sup> showed that when the portal vein of the rat is partially ligated so as to restrict the volume of blood entering the liver, the extent of restoration following removal is greatly reduced. We have noted always marked congestion of the remnant of the liver the first few days after the removal of the usual 70 per cent component. The sinusoids distend in order to make a capillary bed available that is adequate to handle the pre-operative portal blood volume. This distention we believe induces coincident hypertrophy of the hepatic cell which, with the occasional mitosis, may largely explain all that is involved in hepatic restoration.

In the presence of obstruction, however, the marked distention of the sinusoids of the remnant of organ, following removal of the 70 per cent component, did not occur. In some cases, the sinusoids were open and, of course, circulation through the glands ensued, but the rather marked congestion during these early hours following operation was not observed in animals with biliary stasis. This, we feel, is largely due to the distention of the hepatic cells with bile, encroaching somewhat on the sinusoids and impeding the flow of the usual volume of blood.

The changes in the liver that occurred during the fourth week, as determined by the loss of weight as well as by histologic section, are of interest (fig. 1 *b*). If the animals that survived and were killed on the twenty-eighth day after operation had experienced the same hepatic changes as those that were killed up to the end of the third week, and there is every reason to believe that they had, for jaundice of sclera, mucous membranes and urine were well marked, then, with the reestablishment of a patent bile duct, the retained constituents of the bile were removed, and the liver became more nearly normal. Under these conditions, then, the ratios of weight of liver to weight of body recorded at twenty-eight days were not only greatly lower than those at twenty-one days, but they were considerably lower than those describing the extent of restoration in the control animals (fig. 1 *a*).

On the basis of these observations we were led to believe that in the presence of obstruction to the bile duct in white rats, true restoration following the removal of about 70 per cent of the liver is not as marked as in animals in which the bile duct remains intact.

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4. Stephenson, G. W.: Arch. Path., to be published.

# MECONIUM PERITONITIS

## II. A HITHERTO UNDESCRIBED FORM OF INTRA-UTERINE PERFORATION OF A MECKEL'S DIVERTICULUM

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In a recent review of the etiology and pathology of meconium peritonitis, I<sup>1</sup> brought forth a hitherto undescribed cause for spontaneous intra-uterine perforation of the terminal ileum. In a new-born, full-term infant who died twenty-six hours post partum with fever and abdominal distention there was found post mortem an old, fibrous, calcified body meconium peritonitis, on which was superimposed a recent bacterial inflammation. As the cause of both there was present a small perforation in the antimesenteric side of the terminal ileum. Serial sections through the segment of bowel bearing the perforation revealed excessive development of the lymphoid tissue of a Peyer's patch in the bowel adjacent to the perforation, with the presence of deep, penetrating crypts of Lieberkühn in the lymphoid tissue. The sections through the perforation itself showed this same lymphoid tissue with its deeply invading glands becoming so massive as to cause gradual thinning of the muscularis, which at the perforation completely disappeared, so that the tissue reflected about the opening was composed only of lymphoid tissue and mucosa.

It was conceived that in the pathogenesis the deeply invading crypts of Lieberkühn were derived from the antimesenteric diverticula described by Lewis and Thyng<sup>2</sup> as occurring in pig, rabbit and human embryos at 5 to 32 mm. Generally these diverticula disappear, but investigators<sup>3</sup> have shown their occasional normal occurrence in a wide range of animals, including human fetuses and children, as deep intra-lymphoid crypts of Lieberkühn in the terminal ileum, appendix and even colon.

Since these diverticula occur in 5 to 32 mm. embryos, and since the lymphatic tissue does not develop until the embryo reaches a length

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From the Department of Pathology, Cook County Hospital, Dr. R. H. Jaffe, director.

1. Boikan, W. S.: Arch. Path. **9**:1164, 1930.

2. Lewis, F. T., and Thyng, F. W.: Am. J. Anat. **7**:505, 1907-1908.

3. Lauche, A.: Virchows Arch. f. path. Anat. **252**:39, 1924. Klaatsch, cited by Lauche. Lubarsch, O., cited by Lauche. Meyer, R.: Verhandl. d. deutsch. path. Gesellsch. **10**:216, 1906. Orth, J.: ibid. **3**:135, 1901.

of from 240 to 300 mm., the excessive lymphoid hyperplasia was considered as a reaction to these persistent diverticula. The atrophy of the muscularis was considered secondary to the overdevelopment of the lymphoid tissue. The rupture occurred with the development of a positive intra-intestinal pressure associated with the arrival of the meconium in the lower part of the ileum at about the middle of the fourth month.

It was therefore of great interest when in the study of a second case of meconium peritonitis there was found an identical mechanism, operative, however, this time in a Meckel's diverticulum.

In addition, interesting aspects of fetal physiology and pathology were uncovered. It appeared therefore justifiable to report the study of this second case of meconium peritonitis.

#### REPORT OF A CASE

*History.*—The child was a well developed, colored boy delivered normally and spontaneously. The mother was a young colored multipara, who had received long and intense treatment for syphilis.

At birth, herniation was noticed in the umbilical region. As its condition seemed good, surgical intervention was deemed unnecessary. At the first redressing of the cord, a discharge of meconium was noted about the umbilical defect. Twenty-four hours after delivery, the abdomen appeared distended, and there had occurred no movement of the bowels; fifty-one hours after delivery, the child died.

*Postmortem Examination (Dr. R. H. Jaffé).*—A well developed, colored, male infant, dead two hours, was presented. The skin was icteric. The anterior, posterior and mastoid fontanels were open. The pupils were equal and regular; the conjunctivae, icteric. The breasts were engorged; the abdomen was distended 1 fingerbreadth above the level of the thorax. The testicles were in the scrotum. There were collodion-sealed needle wounds at the inner aspect of the thighs, just above the knees. Attached to the right little finger was a pedunculated structure, 11 by 5 by 6 mm., which resembled a terminal phalanx. On the left little finger, in the same position, was a wartlike structure 1 mm. in diameter. In the region of the umbilicus, there was a defect, 15 mm. long and 5 mm. wide. Through this defect, the umbilical vein and the hypogastric arteries herniated. The umbilical vein was thickened up to 8 mm. and covered by dirty, greenish-gray, fibrinous exudate. The hypogastric arteries were similarly thickened up to 6 mm. and also covered by fibrinous material. In the most distal portion of the vein, the lumen was occluded by a slightly adherent, reddish-gray thrombus. The hypogastric arteries were similarly occluded.

The abdominal cavity contained about 30 cc. of yellowish-green, cloudy fluid. The intestinal loops were all matted together and enveloped in thin, fibrinous exudate. Projecting through this fibrinous wall, in the right lower quadrant, was an elevated, buttonlike nodule with everted edges, composed of injected mucosa, in the central portion of which was an ostium, 1 mm. in diameter, from which yellowish-green fecal material exuded. On separating the intestinal loops, it was found that this ostium was situated in the ileum 6 mm. above the ileocecal valve on the antimesenteric border. Opposite the perforation, the bowel became small, measuring 3 mm. in diameter as compared with the 9 mm. diameter of the ileum above the perforation. In the 2.5 cm. between the perforation and the cecum, the ileum



widened gradually to 5 mm. The colon, which was tightly contracted, measured 5 mm. in diameter, and was therefore only a little over one half of the diameter of the normal-sized ileum. This fact is of importance, as it casts a light on the time of the perforation and on the physiologic factors that determined the development of the bowel. The rectum at its anal end suddenly widened to 8 mm. The lumen was patent throughout. Meconium was present in the bowel only above the perforation. Distal to the perforation, only grayish, thick material was found.

The diaphragm over the right dome of the liver presented an area 3 cm. in diameter through which a corresponding node of the liver was herniated. The sac of the hernia did not contain muscle tissue from the diaphragm, but was composed of peritoneum and pleura. Smaller herniations of a similar character (up to 1 cm. in diameter) were found adjacent to the larger one, but did not contain hepatic tissue.

The spleen weighed 25 Gm., was soft and deep purple.

The liver weighed 210 Gm.; the surface was covered by thick, fibrinous exudate. The consistency was soft. The section surface was greenish brown, with the markings obscured. The gallbladder and ducts were normal.

The pancreas weighed 4 Gm.; the duct was patent.

The suprarenal glands weighed 18 Gm.

The kidneys weighed 40 Gm. The surfaces were smooth. The right kidney had a double ureter that formed a common duct 1 cm. above the bladder. Numerous uric acid infarcts were observed in the pyramids. The wall of the urinary bladder was edematous. There was slight injection about the trigon.

The lungs were covered by rather adherent, fibrinous exudate. The section surface was pinkish gray throughout.

The pericardial cavity contained a little clear fluid. The heart weighed 35 Gm.; the foramen ovale was patent.

*Anatomic Diagnosis.*—Meconium peritonitis with terminal secondary bacterial peritonitis; defect in the lower part of the ileum, on the antimesenteric border, communicating with the abdominal cavity; hypoplasia of the ileum and colon distal to the perforation; herniation of a portion of the liver through the diaphragm and of the umbilical veins and hypogastric arteries through the abdominal wall; patent foramen ovale and ductus venosus; pseudopolydactylism.

*Microscopic Examination.*—Sections were made of all the organs. In addition, the entire intestinal tract was sectioned at short intervals. The segment of terminal ileum bearing the perforation was sectioned transversely, each section being 6 microns' thickness and every third section stained. As normal control there served the serial sections through the entire intestine prepared previously from three newborn infants who died of cerebral injuries. Hematoxylin-eosin stains were used throughout.

The sections of all the organs other than the intestines revealed no abnormality other than microscopic corroboration of the presence of meconium peritonitis. There was no gross or microscopic evidence of syphilis in cutaneous, visceral or osseous organs. Adherent to the mesenteries and serous surfaces of all the abdominal organs was a thick layer of a partially organized admixture of meconium and fibrin, infiltrated with numerous macrophages filled with golden-brown pigment, foreign body giant cells, fibroblasts and round cells, and containing numerous free brown and blue granules and clumps. Numerous polymorphonuclear leukocytes were an evidence of the recent acute bacterial inflammation.

The jejunum and ileum presented no abnormalities. The lymphoid follicles were small, and the muscle gaps produced by the entrance of blood vessels were small. The mesenteric vessels were normal.

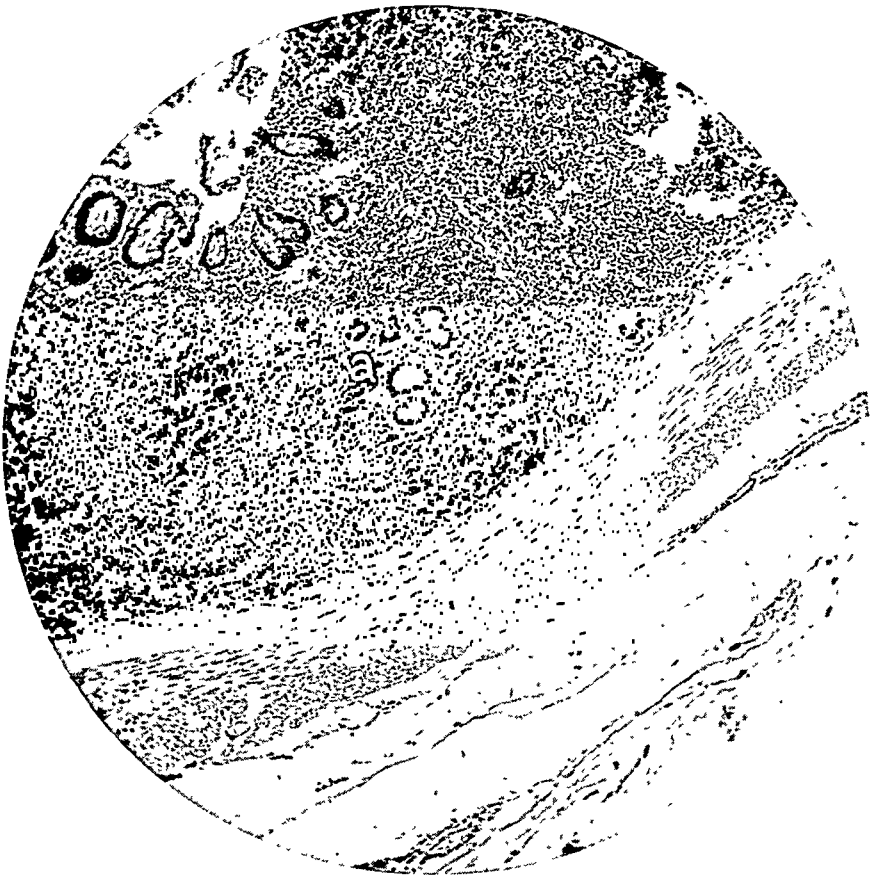


Fig. 1 (level fig. 5 B-1).—The section was made through a Peyer's patch. Note the glands of Lieberkühn deep within the lymphoid tissue in the submucosa (*a*); hematoxylin-eosin;  $\times 72$ .

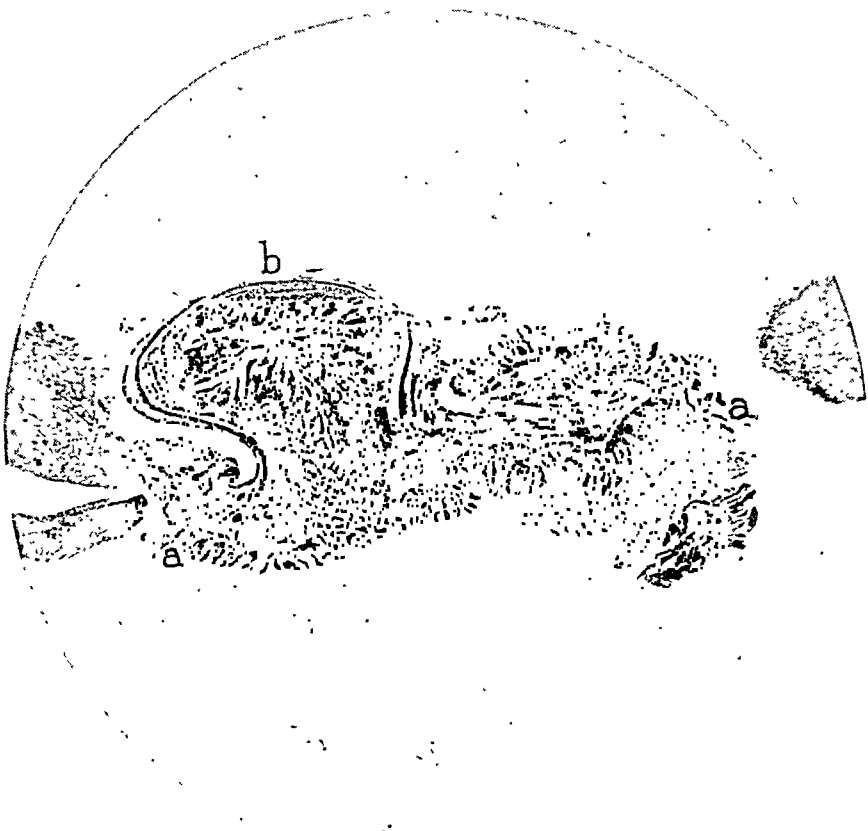


Fig. 2 (level fig. 5 B-2).—This section through the perforation shows the large everted walls of the ruptured diverticulum (*a*); hematoxylin-eosin;  $\times 10$ . At *b* there was an accidental removal of mesentery and muscularis at autopsy.



Fig. 3 (level fig. 5 B-3).—This section shows the huge size of the inferior flap at *a*; in comparison with the total bowel at this level (*b*); hematoxylin-eosin;  $\times 10$ .

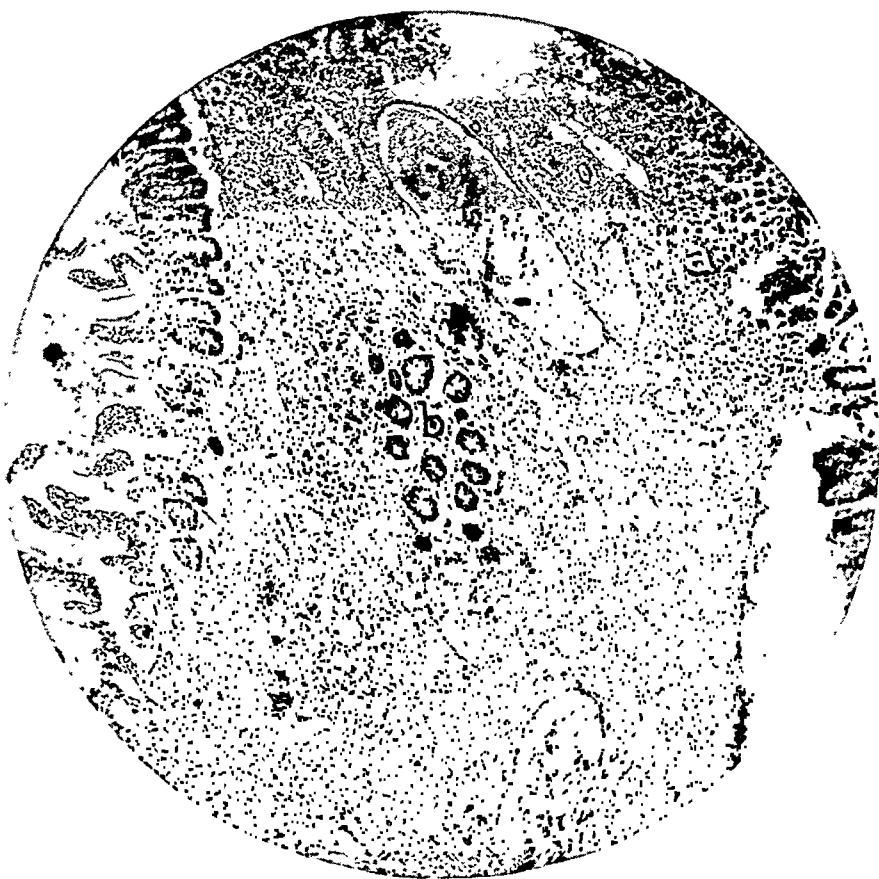


Fig. 4 (fig. 5 B-4).—This section through the distal half of the reflected inferior flap shows the absence of muscularis and the large amount of lymphatic tissue (*a*) and deeply penetrating crypts of Lieberkühn (*b*); hematoxylin-eosin;  $\times 70$ .

The ileum, immediately above the perforation, presented a significant feature (fig. 1; fig. 5 *B-1*). The section was made through a Peyer's patch. The lymphatic tissue composing it extended down to the muscularis and laterally in the submucosa for a considerable extent. The muscularis mucosa was interrupted for the extent of the Peyer's patch, and the crypts of Lieberkühn protruded deep into the lymphoid tissue, to within a short distance of the muscularis. The muscularis was not particularly narrowed in the region of the Peyer's patch. This finding parallels that found in the previous case that I described.<sup>1</sup>

Serial transverse sections through the perforation itself revealed the following: As one approached the perforation, it appeared suddenly on the antimesenteric side and without any upward extrusion of a flap. The bowel at this point diminished suddenly to about one third its size above the perforation. As the serial sections were followed down, two large lateral flaps appeared, which were composed of all the layers of the intestinal wall and were so rolled out as to be completely covered by epithelium. It soon became apparent that the total length of the flaps exceeded

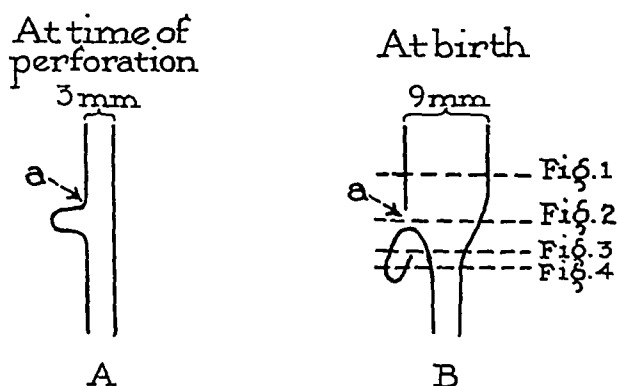


Fig. 5

Fig. 5.—Diagram showing in *A* the diameters of the terminal ileum and the intact diverticulum just preceding perforation, and in *B*, the diameters at time of birth, demonstrating the ruptured diverticulum, the normal development of the proximal (9 mm.) segment and the hypoplasia of the distal segment. The transverse lines refer to the levels from which the sections shown in the preceding four figures were obtained.

the circumference of the segment of bowel from which they arose, a fact incompatible with any conception other than that one was here dealing with the everted walls of a diverticulum, which because of location and position was most likely Meckel's diverticulum (fig. 2; fig. 5 *B-2*). These flaps became progressively larger until at the inferior margin of the perforation they formed a flap that had a width of 7 mm. as compared with the diameter of 3 mm. of the adjacent bowel (fig. 3; fig. 5 *B-3*). Furthermore, this flap was almost completely everted and rolled on itself so as to be completely covered by epithelium, rendering its width at least twice that heretofore mentioned. This inferior flap was tongue-shaped and extended far down alongside the terminal ileum for over 3 mm. At its origin, this inferior flap contained all the layers of the intestinal wall. In its lower two thirds, however, the muscularis rapidly thinned and disappeared, leaving the distal one half of the flap composed of mucosa and submucosa. The latter contained a large amount

of lymphatic tissue and was deeply penetrated by intralymphoid glands of Lieberkühn (fig. 4; fig. 5 B-4). The blood vessels of the flap were widely dilated and filled with blood. In its lower one third, the flap ended tonguelike and was composed of a huge Peyer's patch completely surrounded by mucosa and containing invading Lieberkühn glands.

The epithelium of the diverticulum was of the normal intestinal type. Cystic glands were frequent and were probably of inflammatory origin, from the chemical inflammation in the peritoneum.

Within the segment of the perforation, the bowel was perfectly normal in all respects except for its small size. Distal to the perforation, the ileum was similarly histologically normal with the exception of its small size. It represented, as it were, a bowel uniformly and symmetrically checked in its development. Within the lumen were some shed epithelial cells and irregular débris, but no pigment.

The entire colon formed a 5 mm.-wide, tightly contracted tube—a little more than one half the size of the small intestine. The component layers were histologically normal. The lumen contained a little poorly stained débris and a few cast-off epithelial cells.

#### SUMMARY OF ESSENTIAL FINDINGS

In addition to chronic meconium peritonitis with calcification and fibrosis and recent superimposed acute bacterial inflammation, the essential findings consisted of: A perforation in the antimesenteric side of the ileum, 2.5 cm. from the cecum, bounded by lateral and inferior everted flaps, which far exceeded the size of the perforation and therefore suggested the presence of a perforated, everted Meckel's diverticulum. The lateral flaps consisted of all the layers of the intestinal wall; the lower flap, only in its upper one half, the distal half being composed almost entirely of mucosa and submucosa, the latter occupied by a large amount of lymphatic tissue and deeply penetrating crypts of Lieberkühn. In the ileum, just above the perforation, a Peyer's patch contained Lieberkühn's crypts. Finally, distal to the perforation, the entire bowel was markedly hypoplastic, so that the colon was a little over one-half the size of the small intestine and contained no meconium,

#### COMMENT

The rôle of Meckel's diverticulum in the causation of acute and chronic pathologic abdominal conditions at all stages of intra-uterine and extra-uterine life has been adequately discussed by numerous authors.<sup>4</sup> Meckel's diverticulum is a common cause of fetal intestinal obstruction. Few reports exist as to its spontaneous fetal rupture and

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4. Halstead, A. E.: *Ann. Surg.* **35**:471, 1902. Koch, W., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol. 4, pt. 1, p. 175. Siegmund, H.: *ibid.*, vol. 4, pt. 3, p. 94. Evans, A.: *Brit. J. Surg.* **12**:34, 1929. Christie, A.: *Am. J. Dis. Child.* **42**:544, 1931.

causation of meconium peritonitis. Hunter<sup>5</sup> recently reported gangrene of a Meckel's diverticulum with perforation peritonitis in an infant 4 days old. The cause of the gangrene was considered circulatory. A similar case was reported by Shukowski.<sup>6</sup> Early fetal rupture was described by Genersich<sup>7</sup> and by Orth.<sup>8</sup> In neither case was the histopathogenesis discussed. In analyzing the present case, two questions arise: When did the perforation occur? Why did it occur? They will be discussed in this order.

*Time of Perforation.*—As stated, distal to the perforation the ileum was about one-third the size of the normal intestine above the perforation, and the colon had a diameter of 5 mm. in comparison with the diameter of 9 mm. of the ileum. Now, in its development, the large intestine is for a long time narrower than the small intestine. This is the case even before there is any intestinal content, that is, at about the end of the third month. But the small intestine becomes still wider relatively when, early in the fourth embryonic month, hepatic secretion and formation of meconium commence. Meconium, according to Broman,<sup>9</sup> distends and stimulates the intestine to growth, and its progressive downward propulsion by fetal peristalsis is a most important factor in the proper development of the bowel. At about the end of the fourth month, meconium enters the cecum, and the continuous storage of this material in the large bowel leads to rapid growth of the colon, so that it equals the small intestine in diameter by the seventh or eighth month and then rapidly exceeds it.

The appendix does not take part in this growth, because a valve-like fold of mucosa protects it from the entrance of meconium.

As proof of this conception, Broman cited the stringlike character of the intestine distal to an adhesion. Further, he pointed out that an anomalous communication of the small intestine or cecum that diverts the meconium is almost regularly associated with atresia of the rectum. The latter is, however, untenable, as the anal groove breaks through into the rectum when the embryo is 30 mm., and meconium is not secreted until the embryo is between 90 and 100 mm. This is further borne out in the present case by the absence of any rectal or anal anomalies despite the diversion of meconium. But that meconium does stimulate the development of the bowel is shown in this case, in which diversion of the meconium by the perforation in the lower part of the ileum led to

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5. Hunter, W. C.: *Am. J. Dis. Child.* **35**:438, 1928.

6. Cited by Hunter (footnote 5).

7. Genersich, A.: *Virchows Arch. f. path. Anat.* **126**:485, 1891.

8. Cited by Genersich (footnote 7).

9. Broman, I.: *Normale und abnormale Entwicklung des Menschen*, Wiesbaden, J. F. Bergmann, 1911, p. 344.

marked hypoplasia of the ileum and colon distal to the perforation. Even postnatal gastro-intestinal development is dependent on similar factors. The recent observations of Addis<sup>10</sup> as well as those of others point to an intimate relationship between the length, caliber and weight of the various segments of the gastro-intestinal tract and the character of the contents. He could demonstrate marked changes in rats after forty-four days of a diet high in roughage; the stomach increased 35 per cent in weight, and the colon 48 per cent in weight and 34 per cent in length, with smaller changes in the small intestine. Since observations (Mall<sup>11</sup>) indicate that meconium reaches the cecum at about the fourth month, and since the bowel distal to the perforation contained no meconium, it places the act of perforation at a period near the end of the fourth month.

*Mechanism of Perforation.*—It became obvious that this was a perforated Meckel's diverticulum when it was noted that the flaps about the perforation, when reconstructed, exceeded the size of the diminutive ileum at this point. The perforation of the diverticulum at about the end of the fourth month had diverted the meconium, and the entire bowel distal to the perforation had then ceased to develop.

The etiology of the perforation is to be seen in the serial study of the lower flap, which showed rapid disappearance of the muscle layers, as described in a foregoing paragraph concomitant with the submucosal development of a large amount of lymphatic tissue containing deeply penetrating crypts of Lieberkühn. In its distal third, the flap was composed only of a mass of lymphatic tissue.

In figure 5, *A* represents the bowel and the diverticulum at about the time just before the perforation. *B* represents the bowel as found at autopsy, showing the perforation and the lower flap, the inhibited development of the bowel below the perforation with retention of the same diameter as in *A*, and the normal development of the meconium-filled bowel above the perforation. In *A*, the point marked *a* corresponds to the part of the flap depicted in figure 4. This was a locus of diminished resistance to intra-intestinal pressure, and when the latter became positive by the entrance of meconium, it perforated, causing an eversion of the diverticulum and the formation of the lateral and inferior flaps (fig. 5 *B*).

The thinning and gradual disappearance of the muscularis in the wall of the diverticulum are attributed, as in my previous case, to the excessive development of the lymphatic tissue in the submucosa in association with the deep submucosal intralymphatic crypts of Lieberkühn.

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10. Addis, T.: Am. J. Physiol. **99**:417, 1932.

11. Mall, F. P., in Keibel and Mall: Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, p. 390.

Why this association of excessive development of the lymphatic tissue with intralymphatic crypts of Lieberkühn is not a constant one, —that is, why the latter may be present without excessive development of lymphatic tissue cannot be answered at present, except that in these instances the glands are larger and deeper than in those segments in which there is no excessive lymphoid hyperplasia. Evidence of lymphoid hyperplasia elsewhere in the body was lacking. The distention of the abdomen from the chemical peritonitis was no doubt a factor in the production of the umbilical herniation. The terminal bacterial peritonitis and pleurisy were no doubt of postnatal origin from postnatal bacterial emigration through the intestinal perforation.

#### SUMMARY

A case of meconium peritonitis from spontaneous intra-uterine rupture of a Meckel's diverticulum in the first half of intra-uterine life is described.

The cause of the rupture was the excessive development of lymphatic tissue in association with deep submucosal crypts of Lieberkühn in the wall of the diverticulum, with secondary focal disappearance of muscularis. Rupture took place with the development of positive intra-intestinal pressure from the entrance of meconium.

The short segment of ileum and the entire colon distal to the perforation were hypoplastic and devoid of contents, the colon retaining its early fetal proportion to the small intestine. This is attributed to the lack of the distending and growth-stimulating action of the meconium, diverted by the perforation into the peritoneal cavity.

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# ACTION OF PARATHYROID HORMONE ON THE EPIPHYSEAL JUNCTION OF THE YOUNG RAT

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After Collip described his method for the preparation of highly active parathyroid extracts, many investigations were undertaken to determine the effect of high doses of such extracts. Definite changes were described as occurring when large amounts of parathyroid hormone were administered. These form the pathologic entity of hyperparathyroidism. Furthermore, it has been demonstrated that changes similar to those produced experimentally in animals are found in human subjects in whom a pathologic overfunctioning of the parathyroid glands exists (e. g., hypertrophy or adenoma of the parathyroid glands).

The most striking morphologic effect produced by excessive amounts of parathyroid extracts is the deposition of calcium salts in the soft tissues, such as the kidneys, the heart muscle, the mucous membrane of the stomach, etc. Experimental work has been reported adequately describing these changes in rats<sup>1</sup> and in dogs.<sup>2</sup> That such metastatic calcification may also occur in human subjects as the result of hyperparathyroidism has been shown in a case of parathyroid adenoma combined with general organic calcification which I described.<sup>3</sup>

While hyperparathyroidism leads to a deposition of large amounts of calcium in the tissues, the calcium content of the bones is diminished. An active resorption of bone tissue occurs, and the skeleton becomes weak and brittle, so that numerous spontaneous fractures occur. At the same time, the marrow is transformed into fibrous tissue, so that the changes are very similar to those observed in osteitis fibrosa (von Recklinghausen). This condition is also related to hyperparathyroidism as shown by experiments<sup>4</sup> and observations in man.<sup>5</sup>

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From the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University.

1. Hoff, F., and Homann, E.: *Ztschr. f. d. ges. exper. Med.* **74**:259, 1930.

2. Jaffe, H.; Bodansky, A., and Blair, J. E.: *Klin. Wchnschr.* **9**:1717, 1930.  
Bodansky, A., and Jaffe, H. L.: *J. Exper. Med.* **53**:591, 1931. Jaffe, H. L., and Bodansky, A.: *Proc. Soc. Exper. Biol. & Med.* **27**:795, 1930.

3. Selye, H.: *Med. Klin.* **25**:379, 1929.

4. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: *Proc. Soc. Exper. Biol. & Med.* **27**:710, 1930; **28**:793, 1931; *Arch. Path.* **11**:207, 1931.

5. Hunter, D.: *Proc. Roy. Soc. Med.* **24**:486, 1931. Snapper, I.: *Nederl. tijdschr. v. geneesk.* **2**:5804, 1929. Stearns, J., and Boyd, J. D.: *Proc. Soc. Exper. Biol. & Med.* **26**:717, 1929.

Effects very similar to those produced by parathyroid extracts are also produced by viosterol, the toxicology of which has been studied by many workers during the last few years. This substance likewise leads to metastatic calcification of the soft tissues when given in large doses (Kreitmair and Moll). If large amounts of viosterol are fed to very young animals, spontaneous fractures occur. The rapid skeletal growth of immature animals renders them especially fit for such experiments. Thus I<sup>6</sup> demonstrated that the offspring of rats receiving large amounts of viosterol during the latter half of pregnancy, or only during lactation, show a severe form of osteopsathyrosis, with cessation of growth and numerous spontaneous fractures. These experiments and the later work of others<sup>7</sup> indicate that viosterol can pass through both the placenta and the mammary glands. In somewhat older rats, striking changes in the epiphyseal junctions are produced by an overdosage of viosterol. The zone of cartilage cell columns in these animals is short, and the ground substance between these cells is excessively impregnated with calcium. In adult animals, the changes are not so striking and vary with the diet used.<sup>8</sup>

In their effects on coagulation of the blood, the parathyroid hormone and viosterol have also proved to be closely related. Ample evidence exists to show that both shorten the period necessary for coagulation of the blood.<sup>9</sup>

The similarity of the effects of the viosterol and the parathyroid hormone is striking. In fact, Taylor and his co-workers<sup>10</sup> suggested that viosterol in large doses acts through the parathyroid glands.

The experiments cited show that the bones of very young animals are much more sensitive to the toxic action of viosterol than are those of older animals. Hence it is of some interest to determine the effect of an overdosage of parathyroid hormone on the bones of young rats during the suckling period.

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6. Selye, H.: *Krankheitsforschung* **7**:289, 1929; *Med. Klin.* **24**:1197, 1928.

7. Camel, M.: *Boll. d. Soc. ital. di biol. sper.* **5**:738, 1930. Schoenholz, L.: *Klin. Wchnschr.* **8**:1257, 1929.

8. Schmidtmann, M.: *Virchows Arch. f. path. Anat.* **280**:1, 1931.

9. Selye, H.: *Klin. Wchnschr.* **7**:1891, 1928. Phillips, R. A., and Robertson, D. F.: *Proc. Soc. Exper. Biol. & Med.* **26**:639, 1929. Corson, W. C.; Irwin, G. F., and Phillips, R. A.: *ibid.* **27**:488, 1930. Phillips, R. A.; Robertson, D. F.; Corson, W. C., and Irwin, G. F.: *Ann. Int. Med.* **4**:1134, 1931. Braugher: *Northwest Med.* **29**:38, 1930. Ohshima, Shiro: *Mitt. d. med. Gesellsch. zu Tokio* **43**:115, 1929.

10. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: *Canad. M. A. J.* **24**:763, 1931.

## EXPERIMENTS

Litters of 10 day old rats were used for these experiments. Half of the young rats were treated with parathormone,<sup>11</sup> and the others were retained as controls. Parathormone was administered intraperitoneally, 5 units for each rat daily for three days. The dose was then increased to 10 units daily. The little rats, weighing about 15 Gm. each, tolerated this high dosage relatively well. On the other hand, adult rats given 10 units daily for each 15 Gm. of body weight died within a few days, showing general metastatic calcification. The growth of the young rats treated with parathormone was arrested. About the sixth day of



Fig. 1.—Arrows show points of bending.

the administration of parathormone, a marked deformation of the skeleton became apparent. The diaphyses of the long tubular bones were straight; in the epiphyseal region, however, severe bowing was readily observable. Further treatment resulted in aggravation of these symptoms. The condition produced is illustrated in figure 1.

At autopsy, the bones of these animals were soft and easily cut with a scalpel. The process of ossification of the cartilaginously preformed bones was retarded. While the epiphyses of the normal litter mates were normally ossified almost throughout the whole extent, the ossification centers in the animals treated with parathormone were represented by

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11. The parathormone used in these experiments was provided by Eli Lilly & Company.

only a small area of bone tissue in the center of the cartilaginous epiphyses. The cartilage of the epiphyseal junction was irregular. Shaftward of this cartilage, a red hyperemic line could be seen, and under this a zone of rather dense but soft tissue was visible. It was in this zone that the bending of the bones occurred. The marrow was usually red, but in two cases a pale yellow marrow was found. The calvaria was so thin that only a transparent soft membrane was present. Metastatic calcification in soft tissues occurred only to a limited extent.

Histologically (see figs. 2 and 3), only sections not decalcified were examined. In these, the broad and irregular epiphyseal cartilage was of a pale color in its ground substance. In the zone of provisional calcification, no deposition of calcium could be found in the ground substance in contrast to the findings in the untreated litter mates. There was marked hyperemia in the zone of primary marrow formation corresponding to the red line that was seen macroscopically. Below this line, practically the whole bone consisted of distinctly eosinophilic osteoid tissue. The formation of soft osteoid tissue to so large an extent in this zone seems also to be responsible for the fact that the bones invariably became bent in the region of the epiphyseal junction. The cortex of the bones was thin, and at the point of bending the periosteum invaded the marrow cavity, and there developed a fibrous marrow. In the other parts, however, the bone marrow was chiefly of the lymphoid type.

These findings are especially interesting in connection with the work of Lang<sup>12</sup> and others who, in opposition to Christeller and others,<sup>13</sup> expressed the belief that osteitis fibrosa is only a secondary process, which may occur whenever the bone is so decalcified that the circulation within the bone marrow is subjected to alterations in consequence of mechanical injuries. They also found a close relationship between rickets, osteomalacia and osteitis fibrosa and called attention to the fact that fibrous metaplasia of the marrow occurs also in many cases of human rickets and osteomalacia. In our experiments, the effect of mechanical influences in the production of fibrous marrow was clearly demonstrated, as the fibrous metaplasia always occurred in those areas subjected most to mechanical strain (sites of bending).

The striking changes that are apparent at the epiphyseal junctions as a result of large doses of parathormone, namely, the broad, irregular cartilage, the lack of a deposition of calcium in the zone of provisional

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12. Lang, F. J.: *Klin. Wchnschr.* **5**:228, 1926; *Virchows Arch. f. path. Anat.* **257**:594, 1925; *Beitr. z. path. Anat. u. z. allg. Path.* **87**:143, 1931. Lang, F. J., and Häupl, K.: *Virchows Arch. f. path. Anat.* **262**:383, 1926.

13. Christeller, E.: *Verhandl. d. deutsch. path. Gesellsch.* **21**:7, 1926. Schmorl, G.: *Klin. Wchnschr.* **5**:496, 1926. Stenholm, T.: *Pathologisch-anatomische Studien über die Osteodystrophia Fibrosa*, Upsala, 1926.



Fig. 2.—Normal epiphysis of the control animal: *A*, narrow, regular junction cartilage; *B*, normal bone tissue.



Fig. 3.—Epiphysis of a parathormone-treated litter mate of the animal represented in figure 2. *A*, enlarged, irregular junction cartilage without any trace of a deposition of calcium in the zone of provisional calcification; *B*, osteoid bone tissue; *C*, fibrous marrow.

calcification and the formation of excess osteoid, especially in the region of the cartilage junctions, lead one to view these changes as suggestive of those seen in severe forms of rickets.

Although these changes following the administration of parathormone are suggestive of rickets produced by dietary means, no conclusion as to whether or not the two lesions can be considered identical is possible until further experimental and histologic work now under way is completed.

#### SUMMARY

Young rats are more resistant to an overdosage of parathyroid extract than adult rats. Even when fatal doses of parathormone are administered to lactating rats, extensive metastatic calcification does not occur, as is invariably the rule in adults.

On the other hand, the bony system of young rats is readily and seriously damaged by large doses of parathormone, and the changes so produced are most striking in the region of the epiphyseal junctions in the so-called growth zones. There the changes are *suggestive of severe rickets*.

The fibrous metaplasia of the bone marrow occurs almost exclusively in those areas where severe mechanical strain exists (points of bending of the bones). This corroborates the theory that osteitis fibrosa is not a primary disease of the bone, a pathologic entity, but a secondary effect of mechanical injury to the circulation within the weakened bone, quite independent of the cause of this weakening. Therefore it would appear to be *incorrect to consider osteitis fibrosa simply as a specific effect of hyperparathyroidism*. The occurrence of osteitis fibrosa in the course of experimental scurvy<sup>14</sup> likewise substantiates this view.

The theory that viosterol acts through the medium of the parathyroid glands is not confirmed by these experiments, as the disease produced by hyperparathyroidism in young rats described in this paper, markedly differs from that produced under the same conditions by the so-called "hypervitaminosis D."

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14. Bauer, W.: Beitr. z. path. Anat. u. z. allg. Path. **87**:23, 1931.

# EXPERIMENTAL PRODUCTION OF GALLSTONES

WITH A REVIEW OF THE LITERATURE

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The origin of biliary concretions is still a disputed question. One avenue of information lies in the experimental production of calculi, and numerous attempts have been made to produce them.

Blachstein<sup>1</sup> (1891) injected *B. coli* and *B. typhosus* intravenously into rabbits, and found that those living from eight to one hundred and nine days "usually" contained in the gallbladder opaque yellowish particles consisting of epithelial cells, leukocytes, amorphous masses of bile pigment and clumps of bacteria. He could detect no differences between the effects of *B. coli* and those of *B. typhosus*. Welch<sup>2</sup> could not find these particles in the gallbladder of a rabbit four months after injection of *B. typhosus*.

Marcantonio<sup>3</sup> reported that he infected a dog's gallbladder, but found no formation of calculus after six months. Injection of lactic acid into the gallbladder of a dog gave negative results. He succeeded in producing a kind of gallstone, however, by deposition of pigment calcium on a foreign body placed in the gallbladder of a dog. This deposit contained no cholesterol.

Gilbert and Dominici<sup>4</sup> produced cholecystitis in a rabbit by intravesical injection of *B. typhosus* and found a small concretion in the gallbladder.

Mayer<sup>5</sup> placed pieces of ivory, clay balls and bits of agar in the gallbladders of dogs. After one year, the agar had disappeared, but the solid bodies in some cases had been covered with a thin layer of green-brown or black-green substance, which was pigment calcium and was devoid of cholesterol.

Labes<sup>6</sup> introduced into the gallbladders of dogs irritating, infective, alkaline and acid substances. Of these, the small or soft bodies were

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From the Department of Pathology of the University of Chicago.

1. Blachstein, A. G.: *Bull. Johns Hopkins Hosp.* **2**:96, 1891.

2. Welch, W. H.: *Bull. Johns Hopkins Hosp.* **2**:121, 1891

3. Marcantonio, A.: *Riforma med.*, 1892, vol. 8; cited in *Schmidt's Jahrb.* **238**:13, 1893.

4. Gilbert, A., and Dominici, S. A.: *Compt. rend. Soc. de biol.* **23**:1033, 1893.

5. Mayer, Jacques: *Virchows Arch. f. path. Anat.* **136**:561, 1894.

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expelled, while the larger or harder ones remained in the gallbladder but never became incrustated. Gallstones placed in the viscus dissolved and ultimately disappeared.

Gilbert<sup>7</sup> found a stone in the gallbladder of a dog previously inoculated with *B. coli-communis*.

Mignot<sup>8</sup> was unable to produce stones in the gallbladders of dogs and guinea-pigs by the introduction of foreign bodies. Injection of attenuated strains of *B. coli* resulted in three small biliary calculi in one guinea-pig. He also tamponed the gallbladder of a dog, in the presence of colon bacilli, for one month, after which the tampon was removed. Fourteen months later, the viscus contained from seven to eight facettated stones. These he called cholesterol stones without reporting any analysis.

Richardson<sup>9</sup> injected agglutinated cultures of *B. typhosus* into the gallbladder of a rabbit and succeeded in producing a firm, brown calculus. No analysis of the stone was reported. The same use of an ordinary bouillon culture of *B. typhosus* in another rabbit failed to produce a concretion.

Cushing<sup>10</sup> produced stones in a similar manner, except that he added another factor, intentionally traumatizing the gallbladder by firmly squeezing it between the fingers for some time after the inoculation. He also produced stones up to 3 mm. in diameter by injecting *B. typhosus* into the ear vein of a rabbit accompanied by trauma to the gallbladder. According to Cushing, these stones were composed chiefly of bile pigments combined with calcium. Some of them were coated with cholesterol.

Miyake,<sup>11</sup> working with dogs, was unable to produce stones by infection of the gallbladder with *B. coli*, by tying of the cystic duct or by introduction of foreign bodies into the gallbladder, although the last method resulted in incrustation of the glass pearls or particles of granite with bile pigment. The introduction of foreign bodies into the gallbladder along with infection of the organ gave this same incrustation. Ligating the cystic duct in the presence of an infection with *B. coli*, in a dog, resulted in the formation of two facettated stones after nine months. Foreign bodies in the gallbladder plus infection of it, with ligation of the cystic duct, led to the formation of small stones of calcium, cholesterol and pigment in two dogs. One year after infecting the gallbladder of a rabbit with *B. coli* and narrowing the cystic duct by

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7. Gilbert, A.: Arch. gén. de méd. 2:257, 1898.

8. Mignot, R.: Arch. gén. de méd. 2:129, 1898; Recherches expérimentales et anatomiques sur les cholécystites, Thèse, Paris, 1896.

9. Richardson, M. W.: J. Boston Soc. M. Sc. 3:79, 1898-1899.

10. Cushing, H.: Bull. Johns Hopkins Hosp. 10:166, 1899.

11. Miyake, H.: Mitt. a. d. grenzgeb. d. Med. u. Chir. 6:479, 1900.



placing a piece of gauze under it, Miyake found in the gallbladder a stone which he reported to be rich in cholesterol and containing pigment and much calcium.

Italia<sup>12</sup> reported the production of concretions by intravesical injection of attenuated cultures of *B. typhosus* and *B. coli* into the gallbladders of both dogs and rabbits. The calculi resembled human stones on both gross and microscopic examination, and chemically were shown to contain all the elements found in human stones.

Carmichael<sup>13</sup> inserted a pebble into the gallbladder of a dog and three weeks later injected *B. typhosus* into the organ. After a month, he found a crystalline deposit of calcium carbonate on the pebble. Glass beads left in the gallbladders of rabbits for four and one-half months were covered by hard, sterile masses composed chiefly of carbonate of lime, protein material and bile pigments. A piece of pith, however, unaccompanied by infection, produced no change. Pith infected with either *B. coli* or *B. typhosus* became surrounded by a curdy mass of carbonates and protein material. None of these foreign body coatings contained cholesterol.

Klinkert<sup>14</sup> repeated Cushing's experiments, injecting avirulent typhoid bacilli into the ear vein of the rabbit. In three of nine rabbits gallstones developed composed of organic substance and pigment calcium without cholesterol.

Chalatow<sup>15</sup> fed cholesterol to rabbits in daily doses of from 0.2 to 1.5 Gm. in from 3 to 25 cc. of sunflower seed oil, for from ten to one hundred and forty-two days. Five of the eight fed showed no evidence of formation of stones. The gallbladder of one rabbit contained some sand of crystalline cholesterol after twenty-six days. Another, after eighty-one days, contained considerably more of this sand. In a third, which lived one hundred and forty-two days, he found this same sand and a "large" crystalline concretion resembling a human cholesterol calculus, but without analysis.

Aoyama<sup>16</sup> tied the cystic duct in fourteen rabbits and in three guinea-pigs. He discovered cholesterol concretions in all but six animals. Eight rabbits and three guinea-pigs received four injections of 0.3 Gm. of cholesterol or cholesterol esters in 1 cc. of ether, subcutaneously every other day. The cystic duct was then ligated. Cholesterol concretions were found in all the animals. Feeding of cholesterol by stomach tube daily for from eight to ten days in four rabbits also produced cholesterol stones. The character of the stones was estab-

12. Italia, F. E.: *Policlinico*, 1901; cited, *Zentralbl. f. Chir.* **28**:693, 1901.

13. Carmichael, E. S.: *J. Path. & Bact.* **8**:453, 1903.

14. Klinkert, D.: *Berl. klin. Wchnschr.* **48**:335, 1911.

15. Chaladow, S. S.: *Beitr. z. path. Anat. u. z. allg. Path.* **57**:85, 1913.

16. Aoyama, T.: *Deutsche Ztschr. f. Chir.* **132**:234, 1914.

lished by the crystalline structure, by the solubility in chloroform with reprecipitation in the form of cholesterol crystals, and by the positive color reactions for cholesterol in a chloroform solution of the stones.

Rosenow<sup>17</sup> reported that dogs and rabbits surviving for a long time intravenous injection of streptococci isolated from the gallbladders of patients with cholecystitis not infrequently showed beginning formation of gallstones. He reported later<sup>18</sup> that of eighty animals so treated, six rabbits and three dogs showed definite formation of minute black gallstones, with cholecystitis found constantly. No further description of these stones was given.

Greig<sup>19</sup> found that of eighteen rabbits dying after a long course of intravenous injections of cholera vibrios, nine showed gallstones that, he said, on examination were found to be made up of cholesterol.

Emmerich and Wagner<sup>20</sup> exposed the gallbladders of rabbits and injected 0.5 cc. of a twenty-four hour culture of typhoid bacilli in sodium chloride solution from two to three weeks after immunizing the animals with three doses of typhoid serum. Their animals lived from one hundred to three hundred and forty-one days after operation. Cholecystitis resulted in almost every case, and calculi, measuring from pinhead size to 0.5 cm. in diameter, were found in five of sixteen rabbits. These concretions were considered to be of organic material only, since they burned without residue, showed only a slight cholesterol reaction and gave no pigment reaction.

Dewey<sup>21</sup> injected emulsions of cholesterol intraperitoneally and intravenously into rabbits. One animal receiving 10 cc. of a 2 per cent emulsion intraperitoneally three times a week for forty injections showed no formation of a stone, while another under the same treatment for fifty-two injections showed a few small concretions in the gallbladder. A third received the same injections daily for fifty-eight injections. The gallbladder was filled with small and larger stones. Two rabbits receiving from 5 to 10 cc. of a 1 per cent emulsion, in one case three times a week for sixteen doses and in the other case daily for thirty doses, showed no formation of stones. The combination of thirteen daily intravenous injections with ten daily intraperitoneal injections resulted in the formation of numerous small concretions. Two rabbits received a few intravenous injections followed by a long resting period and then received a few intraperitoneal injections. The gallbladder of one was negative for stones, while that of the other contained numerous

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17. Rosenow, E. C.: *J. A. M. A.* **63**:1835, 1914.

18. Rosenow, E. C.: *J. Infect. Dis.* **19**:527, 1916.

19. Greig, E. D. W.: *Indian J. M. Research* **3**:259 and 397, 1915-1916.

20. Emmerich, and Wagner, G.: *Centralbl. f. allg. Path. u. path. Anat.* **27**:433, 1916.

21. Dewey, K.: *Arch. Int. Med.* **17**:757, 1916.

small concretions. The gallstones were irregular, brittle, not faceted and black. Sections of them examined microscopically showed them to be made up of a framework of disintegrated cells held together by pigment and calcareous substances, forming parallel trabeculae. Chemical examination was not reported.

Venema<sup>22</sup> made typhoid carriers of rabbits by injecting into the gallbladder one-half loop of a twenty-four hour typhoid agar culture in 0.5 cc. of physiologic solution of sodium chloride. In one animal, he found a soft, bright yellow concretion from 3 to 4 mm. in diameter, which was not analyzed.

Sotti and Torri<sup>23</sup> reported that in 1913 they found sterile concretions of definite morphology in the gallbladders of rabbits after splenectomy and ligation of the ductus choledochus. The chemical structure of the stones was not reported.

Iwanaga<sup>24</sup> repeated Aoyama's work. Ligation of the cystic duct, with or without feeding of cholesterol, caused the formation of an amorphous mass in the gallbladder not similar to a gallstone. Subcutaneous injections of cholesterol had no influence on the cholesterol content of the bile and did not cause the formation of these amorphous structures.

Meyer and his co-workers,<sup>25</sup> by intravenous and cystic injection of typhoid bacilli, made rabbits chronic carriers. In the gallbladders of the animals that survived for from one hundred to eight hundred and sixteen days were found, invariably, yellowish-green biliary concretions or blackish calculi, consisting of lime salts with traces of bile pigment and organic material, but with no cholesterol. No calculi were found in the gallbladders of the rabbits living less than thirty days, but they were found in all animals surviving the infection one hundred days or longer.

Badile<sup>26</sup> infected the gallbladders of dogs with *B. coli* and *B. typhosus* after narrowing the ductus choledochus in each with a ligature. Cholecystitis resulted, but there was no evidence of formation of stones. Narrowing the duct and placing gauze or silk thread in the gallbladder caused only slight precipitation on the foreign body. When a fine cholesterol emulsion was introduced into the dogs' gallbladders after the duct in each had been narrowed, small cholesterol

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22. Venema, T. A.: Berl. klin. Wchnschr. **54**:815, 1917.

23. Sotti, G., and Torri, O.: Pathologica **12**:369, 390 and 423, 1920.

24. Iwanaga, H., cited in Centralbl. f. allg. Path. u. path. Anat. **33**:191, 1922.

25. Meyer, K. F.; Neilson, N. M., and Feuisier, M. L.: J. Infect. Dis. **28**:456 and 510, 1921.

26. Badile, L.: Pathologica **15**:307, 1923.

concretions formed. These stones were fragile and gave positive reactions for bile pigment and cholesterol. Microscopically, they showed an amorphous structure inclosing cholesterol granules.

Rous et al.<sup>27</sup> discovered incidentally that small calcium carbonate and calcium bilirubinate stones were formed along the glass and rubber tubings after intubating the gallbladders of dogs for the collection of bile under sterile conditions.

Agrifoglio<sup>28</sup> loosely ligated the common bile duct in dogs, and then injected colon and typhoid bacilli intravenously or into the gallbladder. Concretions formed only in those animals in which attenuated typhoid bacilli had been injected directly into the gallbladder.

Engel and Cserna<sup>29</sup> repeated some of Dewey's experiments. They injected a 2 per cent emulsion of cholesterol daily into the peritoneal cavities of seven rabbits for from four to ten months but were unable to produce gallstones in any case.

Fujimaki<sup>30</sup> observed that in rats fed for a long time on a diet deficient in vitamin A there developed, in sequence, bladder, renal and bile duct calculi. The biliary calculi consisted mainly of cholesterol and pigment. Neither lack of vitamin B nor restriction of the protein seemed to be concerned in such formation of stones.

Stern<sup>31</sup> found no cholesterol coagulation in the gallbladders of rabbits after ten daily intravenous injections of cholesterol.

Hansen<sup>32</sup> fed eight rabbits cholesterol in oil in doses of from 0.5 to 1 Gm. daily for from five to seventy-seven days. In four, cholesterol crystals of microscopic size developed in the gallbladder. One, fed 0.5 Gm. for twelve days, showed three pinpoint-sized pearls of cholesterol. A rabbit that received 10 cc. of a 1 per cent emulsion of cholesterol intraperitoneally once daily for sixty days also showed the cholesterol crystals. Another rabbit that received this same solution subcutaneously, intraperitoneally and intravenously once daily for sixty-six days showed clumps of cholesterol in the gallbladder. The injection of this solution intraperitoneally daily for fifty-six days into a rabbit, which then received cholesterol by stomach tube for eighteen days, resulted in the formation of many pigment kernels and cholesterol crystals in the gallbladder. By narrowing the cystic duct of the gallbladder with a ligature in otherwise normal rabbits, Hansen produced

27. Rous, P.; McMaster, P. D., and Drury, D. R.: *J. Exper. Med.* **39**:77, 1924.

28. Agrifoglio, M.: *Clin. med. ital.* **55**:89, 1924.

29. Engel, K., and Cserna, S.: *Wien. klin. Wchnschr.* **38**:123, 1925.

30. Fujimaki, Y.: *Formation of Urinary and Bile-Duct Calculi in Animals Fed on Experimental Rations*, in Saiki, T.: *Progress of Science of Nutrition in Japan*, League of Nations Health Organization, C. II. 523, Geneva, 1926, p. 369.

31. Stern, R.: *Arch. f. exper. Path. u. Pharmacol.* **112**:129, 1926; **131**:221, 1928.

32. Hansen, S.: *Acta chir. Scandinav.* **62**:483, 1927.

pigment stones without cholesterol content. This same procedure in hypocholesteremic rabbits, however, resulted in almost constant production of cholesterol crystal concretions.

Whitaker<sup>33</sup> reported soft masses in the gallbladders of four of sixteen cats after cutting and dilating the sphincter of the common bile duct.

Copher and Illingworth<sup>34</sup> repeated Whitaker's experiment on eighteen cats, but were unable to confirm his results. They also combined this procedure with the introduction of *B. coli*, staphylococci and streptococci in the gallbladders of cats, but found no cystitis or lithiasis even after several weeks. The same infection in dogs together with ligation of the common duct below the middle hepatic duct gave the same result. Cutting the sphincter in cats together with the introduction of many kinds of foreign bodies into the gallbladder, and in some cases with infection of the viscus, gave negative results. Nor could they produce stones by cauterization of either the mucosa or the serosa of the gallbladder with phenol after cutting the sphincter in cats.

Wilkie,<sup>35</sup> as did Rosenow, injected streptococci isolated from human gallbladders into the lumen of the gallbladder, into the wall of the gallbladder and intravenously in rabbits. The intravesical method gave negative results. The intramural method after three months produced an inflamed gallbladder, which contained pinhead-sized stones composed of large amounts of cholesterol but no calcium. The intravenous method was fruitless until, by repeated injections, chronic cholecystitis was produced, occasionally accompanied by the formation of cholesterol stones without trace of calcium. Injecting the streptococci intramurally with the cystic duct ligated produced stones in some cases. These stones contained a large amount of calcium as well as cholesterol. Repeated intravenous injection of the organisms with the cystic duct ligated resulted in the formation of stones composed mostly of calcium with but relatively little cholesterol.

Whitaker<sup>36</sup> stated that chemical examination of the masses that he produced in 1927 revealed them to be largely blood clots. He then reported masses resembling stones found in seven of nine cats, which he had produced by inducing stasis and hyperconcentration of bile through fasting and dehydration for from five to fifteen days while the animals were kept sleeping peacefully under barbital anesthesia.

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33. Whitaker, L. R.: *J. A. M. A.* **88**:1542, 1927.

34. Copher, G. H., and Illingworth, C. F. W.: *Surg., Gynec. & Obst.* **46**:658, 1928.

35. Wilkie, A. L.: *Brit. J. Surg.* **15**:450, 1928.

36. Whitaker, L. R.: *Surg., Gynec. & Obst.* **48**:396, 1929.

The stones consisted of numerous particles ranging in diameter from that of a grain of dust up to 1 to 2 mm. and varying in consistency from semisolid to solid. No chemical analysis was reported.

Illingworth,<sup>37</sup> studying the "strawberry" gallbladder, reported the production of a large, semisolid concretion in the gallbladder of a single rabbit by feeding 0.2 Gm. of cholesterol daily for thirteen weeks and making an intramural injection of streptococci one week after the beginning of the feeding. No analysis or further description of the stone was reported.

Usuki<sup>38</sup> fed young rabbits a diet deficient in the fat-soluble vitamins for periods of from thirty-five to one hundred and seven days. Of the forty-three animals used, four developed numerous minute (millet seed) concretions.

Westphal and Gleichmann<sup>39</sup> narrowed the cystic duct in dogs for varying periods of time and combined this stasis with feeding of cholesterol and also with injections of animal charcoal and sodium chloride into the gallbladder. They succeeded in producing bilirubin concretions, usually of pinhead size, but in one case measuring up to 1 cm. in diameter. Microscopic examination of the bile from some of the animals fed cholesterol showed globules, presumably of cholesterol, radiating about the surface of minute pigment concretions. No cholesterol stones were found.

In summary, we find that, in the main, five methods have been used to produce calculi: introduction of foreign bodies into the gallbladder, production of stasis in the gallbladder, infection of the organ, induction of hypercholesteremia and production of a deficiency of vitamins. The use of foreign bodies even when combined with infection has resulted only in a coating of the bodies with a layer of pigment, and that only occasionally. Stasis in the gallbladder alone has given no results, except in one instance (Hansen) in which a pigment stone, without cholesterol content, was formed. Infection, alone and with stasis, has resulted in the formation of stones on many occasions. Often these stones were described as cholesterol stones, but in those instances in which analyses were made, the concretions were found to be either pigment stones, with or without calcium carbonate, or organic material consisting chiefly of tissue débris without appreciable cholesterol content. Cholesterol stones have been reported in a few instances in hypercholesteremic animals, and the character of the stones has been substantiated by analysis. Yet the animals in which positive results

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37. Illingworth, C. F. W.: *Brit. J. Surg.* **17**:203, 1929.

38. Usuki, K.: *Jap. J. Gastroenterol.* **1**:18, 1929.

39. Westphal, K., and Gleichmann, F.: *Ztschr. f. klin. Med.* **115**:329, 1931.

were shown numbered but a few of those used in each series, and other investigators have failed to confirm the results. Hypercholesteremia combined with stasis in the gallbladder has given no better success. A few workers have reported finding minute cholesterol stones in vitamin-deficient animals. Too little information is available at this time to come to any conclusion concerning the application of these findings. Strangely, the combination of infection and hypercholes-

*Data of Experiments*

Rabbit	Length of Time Given 2 Injec- tions of Choles- terol Weekly	Length of Time Survived Injections of Typhoid Bacilli	Cholecystitis	Gallbladder Content
1	6 wks.	(No injection)	0	Normal
2	7 wks.	(No injection)	0	Normal
3	2 mos.	(No injection)	0	Normal
4	2 mos.	(No injection)	0	Numerous hard pigment stones up to 1 mm. in diameter
5	2 mos.	(No injection)	0	Cholesterol crystals in the bile
6	4 mos.	(No injection)	0	Many minute pigment granules
7	5 mos.	(No injection)	0	Normal
8	(No cholesterol given)	5 mos.	Definite	Plug of cellular debris
9	(No cholesterol given)	16 mos.	Very definite	Lumen obliterated
10	3 mos.	12 mos.	0	Normal
11	5 mos.	12 mos.	0	Normal
12	5 mos.	5 mos.	0	Clear bile containing numerous minute, hard pigment granules
13	3 mos.	1 mo.	Definite	Cheesy, organic debris
14	4 mos.	3 mos.	Very definite	Organic debris and many hard pigment granules (2 x 1 mm.)
15	6 mos.	3 mos.	Slight	Cellular debris and microscopic hard pigment granules
16	5 mos.	7 mos.	Slight	Soft pigment clumps in bile
17	9 mos.	10 mos.	Slight	Many soft pigment clumps up to 1 mm. in diameter
18	2 mos.	2 mos.	Slight	Organic debris, small pigment granules, microscopic choles- terol crystals
19	6 mos.	11 mos.	Slight	Clumps of soft organic debris and cholesterol crystals in bile
20	5 mos.	3 mos.	Definite	Cellular debris and very numer- ous cholesterol crystals
21	6 mos.	10 mos.	Very definite	Heavy precipitate of choles- terol with 8 pigment clumps up to 3.5 mm. in diameter

teremia has not been extensively tried. Illingworth found his concre-  
tion incidentally. The experimental work that I shall now describe  
concerns an attempt to produce cholesterol gallstones in rabbits by a  
combination of these two factors, continued high blood cholesterol plus  
infection of the gallbladder.

METHOD

A 1 per cent watery emulsion of cholesterol was made according to the method  
described by Dewey.<sup>21</sup> Healthy rabbits each received 10 cc. of this emulsion intra-  
peritoneally twice a week for varying periods up to nine months. The blood  
cholesterol changes were followed, and after the animals had become hypercholes-  
teremic, the abdomen of each was opened, 1 cc. of bile was aspirated from the

gallbladder, and about 1 cc. of a suspension of a twenty-four hour growth of typhoid bacilli was injected into the viscus. To prevent leakage into the peritoneal cavity, the needle was inserted into a tip of the liver, through the thin mesentery of the gallbladder, and thence through the wall of the gallbladder. The bile was aspirated, and the organisms injected through the same needle without removing it between the operations, so that only a single puncture was made. The abdomen was closed, and after two weeks' rest, the injections of cholesterol were continued. The typhoid infection was produced from two to ten weeks after the injections of cholesterol were started, and these injections were continued for from five to thirty-two weeks after the infection. A few of the animals were killed. Some of them died from peritonitis resulting from the intraperitoneal injections. Those surviving the injections were left undisturbed to die of natural causes. These died from one to eight months after cessation of the injections.

#### OBSERVATIONS

Of the seven rabbits receiving injections of cholesterol only, four showed no biliary changes. Only one showed any cholesterol change in the gallbladder, and that was in the form of fine cholesterol crystals in the bile. Strangely, the bile of two rabbits contained black concretions, in one case of pinhead size, in the other measuring up to 1 mm. in diameter. These granules were irregular in outline and very hard. They contained no cholesterol, but were composed of calcium bilirubinate. The wall of the gallbladder was normal in all seven rabbits.

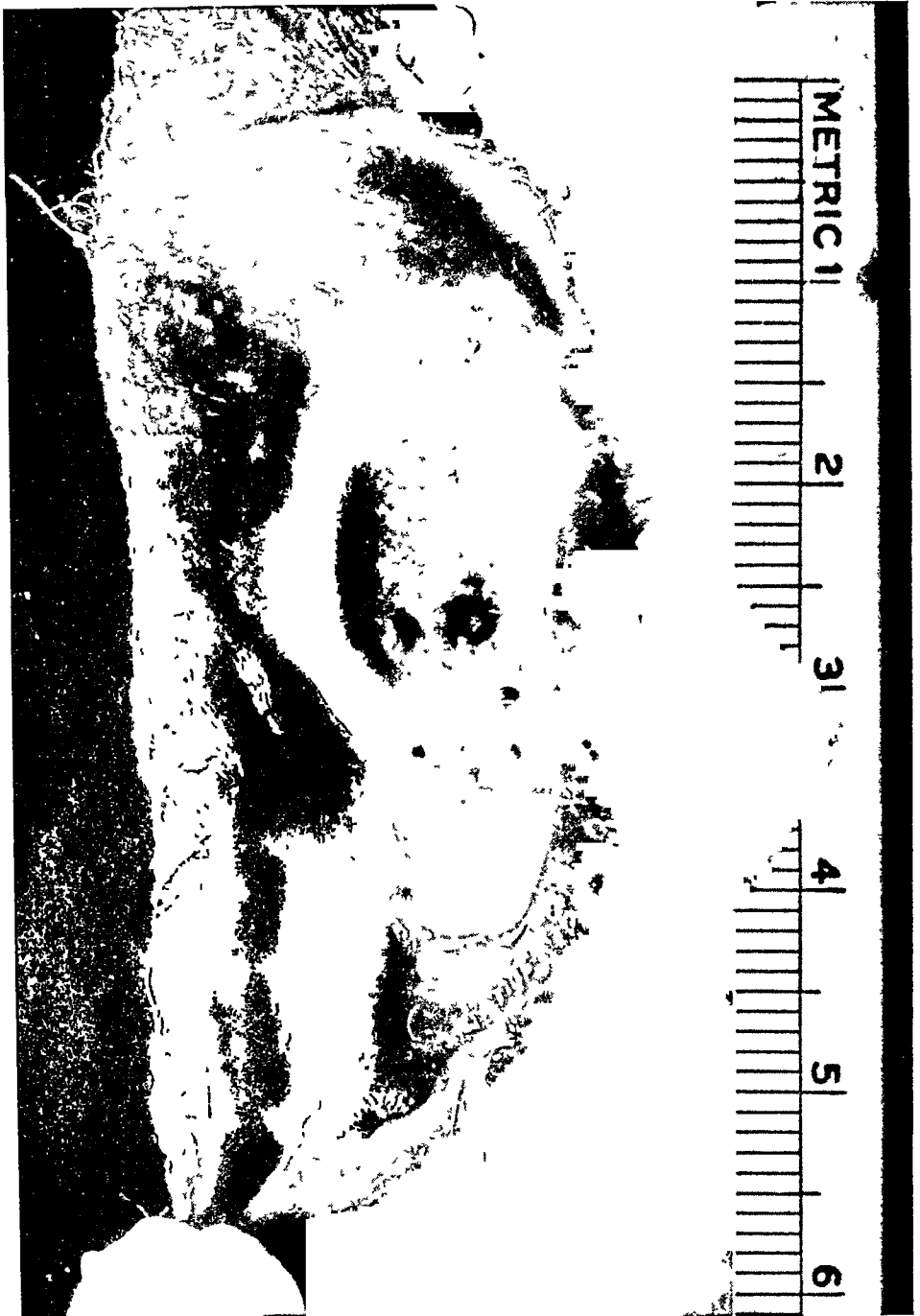
Both of the rabbits (8 and 9) receiving only the intravesical injection of typhoid bacilli showed marked chronic cholecystitis. The wall of the gallbladder was white, opaque and very thick. In the rabbit living five months after infection, the lumen of the viscus contained a plug of white, cellular débris and no bile. The gallbladder of the rabbit killed after sixteen months had a wall measuring 1 mm. in thickness, and the lumen was almost entirely obliterated.

Of the twelve rabbits receiving both the cholesterol and the typhoid injections, three showed a normal wall of the gallbladder with no evidence of infection. The bile was also normal in two of these, but in the third it showed pigment granules similar to those seen in the first group of animals. The other ten showed cholecystitis of varying degrees of intensity. In five there was slight thickening of the wall with a moderate degree of lymphocytic infiltration into the wall, as described in typhoid carriers (Mallory<sup>40</sup>). In two, this process was more severe, and there was some hypertrophy with fibrosis of the wall. The other two showed a very thick wall, composed largely of dense fibrous tissue. All of these ten showed changes in the bile. The bile in each case contained the organic débris seen in the rabbits receiving

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40. Mallory, T. B., and Lawson, G. M.: *Am. J. Path.* 7:71, 1931.





The gallbladder of rabbit 21. The rabbit received biweekly intraperitoneal injections of cholesterol for two months, followed by an intravesical injection of *B. typhosus* and a continuance of the injections of cholesterol for another four months. The animal was killed seven months later. Note the tremendously thickened wall of the gallbladder, the ten pigment concretions in the bile (one lies in the cystic duct, but does not occlude it) and the innumerable flecks of cholesterol present both in the bile and in the mucosa of the gallbladder.

only the typhoid bacilli.. The gallbladders of two of them also contained the hard pigment granules seen in the first group of rabbits receiving only the cholesterol. Six of the rabbits, however, showed further changes in the bile: In four, soft, brown clumps were found that did not resemble the small, hard, black granules of pigment, and at first these were thought to be masses of cholesterol. On analysis, however, the masses were shown to contain only traces of cholesterol and consisted largely of calcium bilirubinate. In two of these rabbits there were also found in the bile large numbers of cholesterol crystals. Two other rabbits showed these crystals, but not the clumps of pigment. Rabbit 21 showed the condition illustrated in the accompanying photograph. The wall of the gallbladder was markedly thickened; the bile was filled with large flakes of yellowish cholesterol and contained ten soft concretions, the largest measuring irregularly 3.5 mm. in diameter. One of the smaller stones can be seen in the beginning of the cystic duct, where it was found lying loosely without occluding the duct. The bile was filled with precipitated cholesterol; yet an alcohol-ether extraction of the concretions showed only a trace of cholesterol on precipitation with digitonin.

#### COMMENT

Why two of seven hypercholesteremic rabbits should show pigment concretions in their gallbladders is unexplained. Hansen<sup>32</sup> described similar findings. The literature contains no reference to spontaneous gallstones in rabbits. Only one of the seven showed cholesterol crystals in the bile. Of the ten hypercholesteremic rabbits with infected gallbladders, four showed cholesterol crystals in the bile, and two of these and two others showed changes in bile pigment not seen in the rabbits with noninfected gallbladders.

Although the method used failed to produce the desired result, cholesterol gallstones, yet it did result in precipitating out of the bile the elements making up gallstones. Many authors believe that cholesterol stones form by accumulation of precipitated cholesterol on bile pigment nuclei. Others feel that organic débris is the nidus. Four of the infected rabbits showed precipitation of cholesterol in the bile, six of them showed pigment nuclei, and all of them showed cellular débris in the bile. Pigment stones had formed, but no great quantity of cholesterol was adherent to the surfaces in the two cases in which both elements were present; nor had the cholesterol coated the cellular débris in the four cases in which the crystals and débris existed together. Whether some additional factor is necessary to lead to accumulation of the cholesterol on the nidus, or whether it is merely a question of greater time required for the growth of the stone, demands further investigation.

## SUMMARY

A combination of hypercholesteremia, induced by intraperitoneal injections of a watery emulsion of cholesterol, and infection of the gallbladder, produced by direct cystic injection of *B. typhosus*, failed to lead to the formation of cholesterol gallstones in rabbits. Pigment calculi were formed in many of the gallbladders, and in some of the rabbits cholesterol crystals were found in the bile. In no instance, however, had any appreciable quantity of the cholesterol been incorporated in or incruled on the pigment stones.

The numerous methods used in attempts to produce gallstones experimentally are given in a review of the literature. Foreign bodies in the gallbladder have frequently become coated with pigment. Stasis alone has been unsuccessful, but when combined with infection has, on occasion, led to the formation of pigment stones often combined with carbonates. Cholesterol stones have been found in hypercholesteremic animals in a few instances, but these results have failed of confirmation. Recently, minute cholesterol stones have been reported in a few vitamin-deficient animals. Various combinations of the methods named have given no better results. No method has been found that will produce gallstones, especially of the cholesterol type, with any degree of certainty.

# General Review

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## THE PATHOLOGIC PHYSIOLOGY OF THE PARATHYROID GLANDS

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AND

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Until recently knowledge of the rôle of the parathyroid glands in health and disease was based almost exclusively on animal experimentation. Such experimentation was stimulated primarily by the experiences of surgeons in cases of "total" thyroidectomy, such as the observance of the frequent occurrence of symptoms of tetany following this operation. In view of the proximity and the close histologic similarity of the parathyroid gland to the thyroid gland, no independent significance was attached to them until Gley<sup>1</sup> made his fundamental experiments. He was the first to show that in rabbits tetany ensued when the parathyroid glands were removed with the thyroid gland, but that tetany did not ensue when the parathyroid glands were left behind. Gley and a number of other observers, finding an enlargement of the parathyroid glands that had been left behind in a thyroidectomy, concluded that these enlarged glands compensated for the function of the removed thyroid gland. The conception of a compensatory rôle of the parathyroid glands was definitely refuted by the work of Vassale and Generali.<sup>2</sup> They found that removal of all parathyroid glands resulted in fatal tetany even when the entire thyroid gland was left behind. When, on the other hand, these investigators removed the entire thyroid gland, but spared one or several of the parathyroid glands, tetany did not develop in the animals. These experiments, corroborated by numerous additional observers, furnished proof of a causal relationship between removal of the parathyroid glands and tetany, and led to the establishment of the symptom complex of "tetania parathyreopriva."

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From the Western Pennsylvania Hospital Institute of Pathology, Ralph R. Mellon, Director.

1. Gley: *Compt. rend. Soc. de biol.*, 1891, pp. 557, 567, 583 and 843.

2. Vassale and Generali: *Riv. di pat. nerv.* **1**:249, 1896; *Arch. ital. di biol.* **25**:459, 1896; **26**:61, 1896.

In dogs, after parathyroidectomy, some of the earliest symptoms of an attack of tetany are loss of appetite and increasing restlessness. Later there appear fine fibrillary contractions of the temporal muscles, then of other muscles of the head and jaw. Recovery from such an attack is often followed by other and more severe attacks at progressively shorter intervals. Death is commonly produced by asphyxia resulting from spastic contraction of the larynx and the muscles of respiration (Dragstedt<sup>3</sup>).

Once the importance of parathyroid function had been demonstrated, the efforts of investigators centered for many years on the problem of the sequelae of parathyroidectomy. It soon became doubtful whether removal of the parathyroid glands was the sole and direct cause of tetany. When animals, subsequent to a partial parathyroidectomy, had recovered from the postoperative tetany, they were found to be in a state of latent tetany. A number of factors would, in such animals, elicit tetany. Among these factors, pregnancy was quite well established. Trauma, psychic trauma, exhaustion and infection were also found to play a rôle. Nutritional factors also are evidently of considerable importance. A diet rich in meat has, in the experience of several investigators, tended to aggravate postoperative tetany, while a diet rich in milk has, in some experiments, served to arrest the fatal consequences of parathyroidectomy. It is apparent from such observations that parathyroidectomy as such does not cause tetany, but increases the nervous irritability to such an extent that normally harmless factors elicit tetany in these animals.

Following a nonfatal parathyroidectomy, chronic pathologic consequences ensue. Cachexia and psychic disturbances have been observed, and symptoms referable to a disturbance of the mineral metabolism have been the object of numerous investigations. The appearance of cataract and the shedding of the hair have been noted.

#### RELATION TO CALCIUM AND PROTEIN METABOLISM

Of outstanding importance have been observations on changes in the teeth following parathyroidectomy. Erdheim<sup>4</sup> first directed attention to chronic sequelae of parathyroidectomy in rats when he found that in these animals the newly formed dentin of the gnawing teeth calcifies either too late and in a faulty way or not at all. In contrast to the physiologically regenerating gnawing-teeth, the other teeth, which were fully developed at the time of the parathyroidectomy, remained unaf-

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3. Dragstedt: *Physiol. Rev.* **7**:499, 1927.

4. Erdheim: *Wien. klin. Wchnschr.* **19**:716 and 817, 1906.

fect. Erdheim's observations were amplified and fully corroborated by Toyofuku.<sup>5</sup> It became apparent that the disturbance of calcium metabolism subsequent to removal of the parathyroid glands is not restricted to the dentin of growing teeth. Erdheim<sup>6</sup> was able to show that in these animals, young or adult, fractures of the bones heal by the slow formation of a callus that is remarkably poor in calcium salts. He compared these findings with those in human rickets and osteomalacia. Chemical analyses of the bones of such animals were carried out by Ogawa,<sup>7</sup> who found a decrease in calcium of the bones, particularly marked in cases in which tetany developed. A decrease in calcium subsequent to parathyroidectomy was further observed in the brain by MacCallum and Voegtlin<sup>8</sup> and in the blood serum by MacCallum, Hastings and Murray,<sup>9</sup> and by many other investigators. On the other hand, an increase in the excretion of calcium through the urine of these animals could not be corroborated by Cooke.<sup>11</sup> Although a number of investigators were unable to find a decrease in calcium in the serum and central nervous system of the animals operated on, these negative results do not refute the importance of the calcium metabolism among the sequelae of parathyroidectomy.

From the researches of Loeb<sup>12</sup> it is known that muscular irritability depends on the quantitative relation between calcium ions on the one hand and potassium and magnesium on the other. A relative decrease in the number of calcium ions leads to muscular spasms. When we consider that, according to Rona and Takahashi,<sup>13</sup> only one fifth of the total calcium of the blood is present as calcium ions, it becomes clear that even in the absence of a decrease in the total serum calcium the number of calcium ions may be decreased. The significance of the calcium ion concentration of the blood was demonstrated by the experiments of MacCallum, Lambert and Vogel.<sup>14</sup> They removed the calcium ions from blood by dialysis. This blood then induced muscular spasms; on addition of calcium, it lost its spastic effect. Using the frog's heart as an indicator for the calcium ion concentration, Trendelenburg and

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5. Toyofuku: *Frankfurt. Ztschr. f. Path.* **7**:249, 1911.

6. Erdheim: *Frankfurt. Ztschr. f. Path.* **7**:175, 1911.

7. Ogawa: *Arch. f. exper. Path. u. Pharmakol.* **109**:83, 1925.

8. MacCallum and Voegtlin: *J. Exper. Med.* **11**:118, 1909; *Bull. Johns Hopkins Hosp.* **19**:91, 1908.

9. Hastings and Murray: *J. Biol. Chem.* **46**:233, 1921.

10. Footnote deleted by the author.

11. Cooke: *J. Exper. Med.* **21**:45, 1910.

12. Loeb: *Am. J. Physiol.* **3**:327, 1900.

13. Rona and Takahashi: *Biochem. Ztschr.* **31**:336, 1911.

14. MacCallum; Lambert, and Vogel: *J. Exper. Med.* **20**:149, 1914.

Goebel<sup>15</sup> found a definite decrease in the number of the calcium ions in the serum of cats subsequent to parathyroidectomy.

The inference, then, that the removal of the parathyroid glands leads to a disturbance of the calcium metabolism appears justified. Whether this metabolic disturbance is directly or indirectly referable to the parathyroidectomy is still somewhat a matter of dispute. A decrease in the H-ion concentration in tetany was pointed out by Wilson, Stearns and Janney<sup>16</sup> and others—an observation that may lead one to interpret the decreased calcium concentration of the blood after parathyroidectomy as due to "alkalosis" of the blood. A number of observers stress the importance of increased phosphorus content of the serum subsequent to removal of the parathyroid glands (Greenwald<sup>17</sup> and others), since this increase in phosphorus would also in itself lead to a decrease in the calcium ion concentration.

In the opinion of some workers, parathyroidectomy leads primarily to a disturbance of intermediate protein metabolism. The latter, in turn, through the resulting alkalosis, induces a decrease in the calcium ion concentration. This contention appears to be based principally on the following facts: Koch<sup>18</sup> found that following parathyroidectomy certain protein split products, the guanidines, appear in increased quantity in the urine. His observations were corroborated by those of Paton and Findlay<sup>19</sup> and others. As further supporting this view, the guanidine contents of the muscles were found diminished by Henderson.<sup>20</sup> In addition, Paton,<sup>21</sup> experimenting with cats, found that the symptoms of guanidine poisoning were highly analogous to those of "tetania parathyreopriva." In particular, he pointed out that the animals showed the same type of electric hyperirritability that is characteristic of tetany after removal of the parathyroid glands. The work of Paton and Findlay,<sup>19</sup> and that of Herxheimer,<sup>22</sup> on guanidine poisoning after parathyroidectomy led these authors to conclude that subsequent to this operation the susceptibility to the effects of guanidine is doubled.

On the basis of the observations on guanidine in animals after removal of the parathyroid glands, the resulting tetany has been

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15. Trendelenburg and Goebel: *Arch. f. exper. Path. u. Pharmacol.* **89**:171, 1921.

16. Wilson; Stearns, and Janney: *J. Biol. Chem.* **23**:89, 1915.

17. Greenwald: *Am. J. Physiol.* **28**:103, 1911; *Biochem. Ztschr.* **54**:159, 1913.

18. Koch: *J. Biol. Chem.* **12**:313, 1912.

19. Paton and Findlay: *Quart. J. Exper. Physiol.* **10**:315, 1916.

20. Henderson: *J. Gen. Physiol.* **52**:1, 1918.

21. Footnote deleted by the author.

22. Herxheimer, in Henke and Lubarsch: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 8, p. 548.

interpreted as guanidine poisoning (Biedl<sup>23</sup>). It was thought that parathyroid insufficiency, through an increased effect of guanidine, led to alkalosis and that the latter, bringing about a decrease in calcium ion concentration, then led to hyperirritability of the nervous system (Herxheimer<sup>22</sup>). The findings of Berkeley and Beebe<sup>24</sup> point in a similar direction. These investigators observed that after parathyroidectomy the administration of calcium was of no therapeutic avail, especially when the animals were put on a meat diet.

Analogies appear to exist between the sequelae of parathyroidectomy on the one hand and symptoms associated with spontaneous hemorrhages into the parathyroid glands of children on the other hand. The considerable frequency of hemorrhage into the marginal parts of the parathyroid glands of children under 5 years of age was pointed out by Yanase,<sup>25</sup> who found its occurrence in thirty-three of eighty-nine cases. He had occasion to examine thirteen children who during life had shown a normal electric irritability, but in none of these did he observe parathyroid hemorrhage. On examination of seventeen children under 1 year of age, who had shown electric hyperirritability, parathyroid hemorrhage was seen in every case. With thirteen children over 1 year, who also had shown electric hyperirritability, parathyroid hemorrhage was found only in three cases. From the investigations of Yanase and others, we infer that a relationship may exist between electric hyperirritability and disturbances of parathyroid growth resulting from hemorrhage rather than the parathyroid hemorrhage as such. However, workers on this subject do not agree that the incidence of parathyroid hemorrhage with electric hyperirritability is actually higher than without such hyperirritability as claimed by Yanase.

#### THE SPECIFIC HORMONE OF THE PARATHYROID GLANDS

Knowledge as to the physiology of the parathyroid glands has been gained primarily by the study of the sequelae of parathyroidectomy, as briefly summarized in the preceding section. This information led to therapeutic attempts. One tried to combat parathyroid insufficiency through supplying artificially the missing internal secretion of the gland. While it had not been possible to extract from the parathyroid gland a regularly effective endocrine principle prior to the recent work of Hanson<sup>26</sup> and Collip,<sup>27</sup> early workers made successful use of transplantation of the parathyroid gland. Pool<sup>28</sup> was the first to transplant

23. Biedl: *Innere Sekretion*, Vienna, 1922.

24. Berkeley and Beebe: *J. M. Research* **20**:149, 1909.

25. Yanase: *Jahrb. f. Kinderh.* **67**:45, 1908.

26. Hanson: *Mil. Surgeon* **54**:218, 1924.

27. Collip: *J. Biol. Chem.* **63**:461, 1925.

28. Pool: *Ann. Surg.* **46**:507, 1907.



a human parathyroid gland in a case of tetany after strumectomy. The success of such transplantation was found to be transitory by numerous workers. Eiselsberg<sup>29</sup> in 1922 gave the number of successful transplantations in man to that time as twenty.

In view of the transitory results attained, most investigators arrived at the conclusion that the transplanted parathyroid gland would, after a certain time, cease to function, and that thereafter recurrences were bound to occur unless the other parathyroid glands regained their function. This inference is supported by the results of animal experimentation on the subject. Observers have reached the consensus that heterotransplantation is less frequently successful than autotransplantation. Some workers even found successful transplantation between individuals of the same species impossible and were able to perform only autotransplantations (Halsted<sup>30</sup>).

The latter experimental procedure furnished ample proof of the function of a transplanted parathyroid gland. When one transplanted part of the parathyroid glands and allowed for sufficient time for the transplants to regain a blood supply, one could then remove all the parathyroid glands that had been left in their normal position, without danger of tetany. When one subsequently removed the transplants, tetany regularly ensued. The influence of transplanted parathyroid glands on the calcium metabolism of teeth was demonstrated by Erdheim,<sup>31</sup> who performed autotransplantations in rats. He found that in the dentin of the gnawing teeth of these animals, a calcium-free zone appeared that corresponded to the time of temporary parathyroid insufficiency subsequent to the operation.

Attempts to combat parathyroid insufficiency by the administration of the parathyroid substance itself or extracts from it have for a considerable time met with partial success. Such extracts as the "parathyroidin" of Vassale were tried in experiments on animals and were found by some to have a beneficial effect on the tetany parathyreopriva when given intravenously in large quantities. Others, however, were unable to corroborate such a therapeutic effect. The extract of Beebe—a nucleoproteid—was found by MacCallum to suppress tetany in animals for some time. Pool<sup>28</sup> saw good results from this extract in human tetany after strumectomy. Biedl<sup>23</sup> in 1922 stated that he had found but two effective parathyroid extracts.

The work of Hanson<sup>26</sup> and Collip<sup>27</sup> led to the preparation of an active parathyroid extract. Injections of this "parathormone" abolish tetany in parathyroidectomized dogs. Application of parathormone leads

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29. Eiselsberg: *Wien. klin. Wchnschr.* **35**:1, 1922.

30. Halsted: *J. Exper. Med.* **11**:175, 1909.

31. Erdheim: *Frankfurt. Ztschr. f. Path.* **7**:295, 1911.

to a rise in the calcium level of the serum. An effect of parathormone on the phosphorus metabolism of animals possibly precedes that on the calcium metabolism. Injection of parathormone leads to an abrupt rise in the urinary excretion of phosphorus without affecting the fecal excretion. At the same time the level of inorganic serum phosphorus is lowered. Collip<sup>27</sup> found that when the serum calcium has attained a certain level, further injections of parathormone bring about an abrupt rise in the serum phosphorus. Greenwald and Gross<sup>32</sup> observed that in normal dogs the rise in serum calcium resulting from injection of parathormone was followed by increased excretion of calcium.

Increased excretion of calcium was also stressed by Hunter and Aub.<sup>33</sup> These observers put patients suffering from lead poisoning on a diet poor in calcium. In these patients a negative calcium balance developed. When parathormone was then injected, an increase of the negative calcium balance ensued. Hunter and Aub concluded that parathormone administered during deficient intake of calcium causes increased excretion of calcium salts from the bones. On the contrary, Ogawa<sup>7</sup> reported an increase in calcium salts of the bones on feeding parathyroidectomized animals with parathyroid substance. The effect of prolonged application of parathyroid extract in animals, particularly in regard to calcium storage, has been studied by a number of investigators in the hope of gaining information on hyperparathyroidism in man. *A priori* the results of animal experimentation must be dependent on the calcium content of the diet as well as on the amount of calcium absorption from the intestinal tract. Indeed, the latter point has already been indicated in the conclusions of Hunter and Aub.<sup>33</sup> The significance of calcium absorption is quite apparent in the results of Morgan and Garrison,<sup>34</sup> who gave dogs cod liver oil or viosterol. On injection of parathormone, the animals presented a rise in serum calcium definitely higher than that in dogs which were not given cod liver oil or viosterol.

Therefore in the study of the effects of injections of parathormone it may be advisable to refer to the results as sequelae of a disturbed correlation between calcium intake and parathyroid function, rather than of a hyperparathyroidism as such. That fatal sequelae opposed to those resulting from parathyroidectomy may follow overdosage of parathormone has been shown by Collip.<sup>27</sup> When this investigator, by injections of parathormone, raised the serum calcium of dogs to 21 mg. per hundred cubic centimeters they presented dulness within twenty-four hours, drowsiness, atonia and failing circulation, and frequently died

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32. Greenwald and Gross: J. Biol. Chem. **66**:185, 1925.

33. Hunter and Aub: Quart. J. Med. **20**:123, 1926-1927.

34. Morgan and Garrison: J. Biol. Chem. **85**:687, 1930.

within forty-eight hours after the first dose. Bauer, Aub and Albright<sup>35</sup> injected increasing doses of parathormone into rabbits and cats for ninety-one days, raising the serum calcium to 15 mg. per hundred cubic centimeters. On gross examination, the spongiosa of the long bones of these animals showed decreased density. It appears noteworthy that these authors also were able to induce decreased density of the spongiosa through a low calcium diet alone, and increased density through a high calcium diet.

Hueper,<sup>36</sup> after prolonged overdosage of parathormone in dogs, saw metastatic calcification in the kidneys, lungs, myocardium and gastrointestinal tract. Jaffé, Bodansky and Blair<sup>37</sup> injected parathormone into dogs and guinea-pigs, into the former for as long as six months. They were able to produce, in this manner, the typical picture of a generalized "ostitis fibrosa cystica." This condition is characterized by general osteoporosis, lacunar resorption of bone, apposition of new bone, fibrosis of the marrow and formation of giant cell "tumors" of bones, probably largely composed of osteoclasts and cysts. The disturbance of the calcium storage of the bones in these experimental animals of Jaffé does not appear to differ fundamentally from that obtained through a diet poor in calcium; in both cases, the investigators induced generalized osteoporosis. The other features of osteitis fibrosa cystica, however, could not be induced by a diet low in calcium. The latter did enhance the effect of the injections of parathormone.

The experiments of Jaffé, Bodansky and Blair have thrown light on the question of the pathogenesis of osteitis fibrosa cystica. Given a certain intake of calcium, parathyroid hormone present in the blood to excess leads to removal of calcium from the bones and the sequelae characteristic of generalized osteitis fibrosa. Discontinuance of the injections of parathormone led, with Jaffé and others, to a reversal of the process, i. e., deposition of calcium in the bones, drop in serum calcium, increase in serum phosphorus.

Several investigators have paid considerable attention to the fact that in cases of general osteitis fibrosa, as well as after injections of parathormone (Jaffé), the blood plasma shows an increase in phosphatase (Hunter,<sup>38</sup> Bodansky and Jaffé<sup>39</sup>). Phosphatase, according to Kay,<sup>40</sup> is an enzyme present in bone extracts that hydrolyzes soluble calcium salts of phosphoric esters to give insoluble calcium phosphate.

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35. Bauer; Aub, and Albright: J. Exper. Med. **49**:145, 1929.

36. Hueper: Arch. Path. **3**:14, 1927.

37. Jaffé and Bodansky: J. Exper. Med. **52**:669, 1930. Blair: Arch. Path. **11**:207, 1931.

38. Hunter: Quart. J. Med. **95**:383, 1931.

39. Bodansky and Jaffé: J. Biol. Chem. **92**:16, 1931.

40. Kay: Brit. J. Exper. Path. **10**:253, 1929; J. Biol. Chem. **89**:267, 1930.

It is present in growing bone in the localities in which deposition of calcium phosphates is most rapidly proceeding. It is absent in cartilage. Robison<sup>41</sup> stressed the rôle of phosphatase in the formation, maintenance and repair of bone. Whether the increase of plasma phosphatase subsequent to injections of parathormone precedes the removal of calcium from bone or is merely a consequence of the regressive changes in bone remains unanswered at present.

RELATION OF THE PARATHYROID GLANDS TO CERTAIN DISORDERS  
OF NUTRITION—OSTEOMALACIA, OSTEOPOROSIS, OSTEITIS  
FIBROSA, ETC.

The reproduction of generalized osteitis fibrosa cystica by Jaffé and his co-workers has added significance to the frequent coincidence of this disease with parathyroid hyperplasia. The significance of this coincidence appears further enhanced by the fact that increase in serum calcium, decrease in serum phosphorus and a negative calcium balance have been found in these cases by a number of investigators (Barr, Bulger and Dixon<sup>42</sup>; Hannon and others<sup>43</sup>; Bauer and others<sup>44</sup>). It appears that as a rule only one or several of the parathyroid glands show hyperplasia. On the whole, the microscopic structure of the hyperplastic gland does not deviate from that of the normal gland. However, it is frequently noted that only a small number of fat granules are present in the hyperplastic gland—a finding that is analogous to that in cases of osteomalacia and that is physiologically typical for the parathyroid gland of the new-born infant (Molineus<sup>45</sup>).

While several observers found in the hyperplastic glands irregularities of the cellular architecture suggestive of atypical proliferation, (Paltauf,<sup>46</sup> Wellbrock<sup>47</sup>), it is noteworthy that in no case of osteitis fibrosa has a neoplastic nature of the enlargement of the gland been demonstrated beyond doubt. We are confronted here with the same difficulty as with other endocrine glands. The neoplastic nature, e. g., of adenoma of the thyroid gland, like that of adenoma of the parathyroid glands, has never been proved and has even been refuted. If a tumor distinctly differs histologically from the normal parathyroid gland, and if a normal parathyroid gland is present in addition to the tumor,

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41. Robison: *Biochem. J.* **17**:286, 1923; **20**:388, 1926.

42. Barr; Bulger, and Dixon: *J. A. M. A.* **92**:951, 1929; *Am. J. M. Sc.* **179**:449, 1930.

43. Hannon; Shorr; McClellan, and DuBois: *J. Clin. Investigation* **8**:215, 1930.

44. Bauer; Albright, and Aub: *J. Clin. Investigation* **8**:225, 1930.

45. Molineus: *Arch. f. klin. Chir.* **101**:333, 1913.

46. Paltauf: *Zentralbl. f. allg. Path. u. path. Anat.* **24**:959, 1913.

47. Wellbrock: *Endocrinology* **13**:285, 1929.

then the latter may justly be referred to as an "adenoma." Such cases have been observed but rarely.

Hoffheinz,<sup>48</sup> reviewing cases of enlargement of the parathyroid glands, found generalized osteitis fibrosa to have been present in seventeen, and osteomalacia in eight, of forty-five cases. It appears, then, that parathyroid hyperplasia is associated with generalized osteitis fibrosa cystica or with osteomalacia in about 50 per cent of the cases. On the other hand, osteitis fibrosa cystica occurs frequently without parathyroid hyperplasia, as was pointed out by Stenholm.<sup>49</sup> In but two of nine cases of this bone disease did Stenholm find hyperplasia of one of the parathyroid glands. On consideration of the fact that the incidence of parathyroid hyperplasia is higher in cases of osteitis fibrosa and osteomalacia than in normal persons of corresponding age, it is safe to infer that some pathogenic relationship exists between parathyroid hyperplasia and those general bone diseases. In regard to the nature of this pathogenic relationship, the anatomic facts allow one to infer that parathyroid hyperplasia does not necessarily lead to the disorders of the bones referred to. Its rôle in the pathogenesis of osteitis fibrosa cystica and of osteomalacia is evidently dependent on further causative factors and may possibly be dispensable.

Jaffé and his co-workers proved in experiments on animals that an excess of parathormone in the blood may lead to osteitis fibrosa cystica. It is, of course, not permissible to conclude from these experiments that an excess of parathormone is the only etiologic factor in osteitis fibrosa cystica. The effect of parathormone on the calcium metabolism of bones depends in part on the total intake of calcium, on the amount of calcium present in the food and on an adequate supply of vitamin D. The interdependence of these three factors, parathormone, supply of vitamin D and intake of calcium, in their effect on the calcium metabolism has been demonstrated by a number of investigators previously referred to (Hunter and Aub,<sup>33</sup> Morgan and Garrison<sup>34</sup>). A primary disturbance of any one of these factors may lead to a secondary disturbance of either of the other two. Inadequate calcium intake has, with Bauer, Aub and Albright,<sup>35</sup> led to decreased density of bones; with Marine,<sup>50</sup> Sorour<sup>51</sup> and Luce,<sup>52</sup> it has been followed by the appearance of parathyroid hyperplasia.

48. Hoffheinz: *Virchows Arch. f. path. Anat.* **256**:705, 1925.

49. Stenholm: *Pathologisch-anatomische Studien über die Osteodystrophia fibrosa*, Uppsala, 1924.

50. Marine: *Proc. Soc. Exper. Biol. & Med.* **11**:117, 1913.

51. Sorour: *Beitr. z. path. Anat. u. z. allg. Path.* **71**:467, 1923.

52. Luce: *J. Path. & Bact.* **26**:200, 1923.

Chicks growing in light from which the ultraviolet portion has been removed acquired, in the experience of Higgins and Sheard,<sup>53</sup> hyperplasia of the parathyroid glands. Administration of cod liver oil prevented this hyperplasia. In view of these experimental data it appears possible that osteomalacia, osteitis fibrosa and related bone diseases are sequelae of a primary metabolic disorder involving the intake of calcium or the supply of vitamin D, or both of these factors. Hyperparathyroidism may then be a link in a chain of events starting with a primary metabolic disorder and finally leading to the characteristic picture of osteitis fibrosa cystica. Whether in the pathogenesis of osteitis fibrosa cystica, hyperparathyroidism is an indispensable or dispensable link—a reciprocal aggravating effect—remains unanswered at present.

In view of the frequent association of osteitis fibrosa cystica with parathyroid hyperplasia, removal of the hyperplastic parathyroid glands has been suggested as a therapeutic procedure. A consensus apparently prevails in regard to the immediate beneficial effect of the operation. A drop of the serum calcium to or below the normal level regularly ensues, and the patient's general condition improves. A diversity of opinion, however, exists as yet in regard to the permanence of this effect. Mandl,<sup>54</sup> who was the first to perform parathyroidectomy in a case of osteitis fibrosa, reported that three and one-half years after the operation the patient was able to walk on crutches and was free from pains. His serum calcium, however, was from 13 to 14 mg. per hundred cubic centimeters, and the x-ray pictures did not show increased density of the bones.

Improvements of lesser duration have since been reported by several workers (Dubois, quoted by Wilder<sup>55</sup>) even subsequent to the removal of normal-appearing parathyroid glands. Wilder,<sup>55</sup> in a case of diffuse fibrous osteitis, saw rapid improvement after removal of an adenoma of a parathyroid gland. Eighteen months later, like Mandl, he noticed that the density of the bones was still far from normal. The dependence of the disease on other than purely endocrine factors was strikingly suggested in Wilder's case: When the therapeutic use of cod liver oil and calcium phosphate was discontinued, the condition of the patient again became worse. Wilder arrived at the inference that his patient was "still suffering from the same disease." One may conclude that the pathogenic importance of hyperparathyroidism for osteitis fibrosa cystica, as indicated by the experiments of Jaffé and others, has been corroborated by the beneficial effects of the removal of enlarged parathyroid glands in this disease. Therapeutic experience has, how-

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53. Higgins and Sheard: *Am. J. Physiol.* **85**:299, 1928.

54. Mandl: *Arch. f. klin. Chir.* **143**:1, 1926.

55. Wilder: *Endocrinology* **13**:231, 1929.

ever, not decisively proved the primary etiologic rôle of hyperparathyroidism in diffuse osteitis fibrosa cystica.

The problem of the sequelae of hyperactivity of the parathyroid glands has been found to be of importance in regard to the pathogenesis of several other diseases of the osseous system. Rats affected with spontaneous rickets were studied by Erdheim.<sup>56</sup> He prepared wax models of the parathyroid glands of these animals and observed a marked enlargement of all these glands. The author interpreted this parathyroid hyperplasia as a consequence and not as the cause of the bone disease. Ritter,<sup>57</sup> studying the parathyroid glands in twelve cases of human rickets, observed hyperemia, edema and sometimes an increase in fibrous tissue. In cases of long duration he found the glands enlarged, but not in more acute cases. These results, like those of Erdheim, pointed to the parathyroid enlargement as a sequel rather than as a cause of rickets. Some workers were unable to find any change of the parathyroid glands in human rickets.

In five of six cases of osteomalacia, Erdheim<sup>58</sup> found either general or focal hyperplasia of the parathyroid glands. He laid stress on the fact that hyperplastic areas, like the parathyroid glands of the newborn infant, contain scarcely any fat granules. Owing to this scarcity of fat, the newly formed glandular tissue stands out clearly from the adjoining parathyroid substance. Erdheim's findings have been corroborated by others. Todyo<sup>59</sup> found hyperplasia of the parathyroid glands in six of seven cases. Among twenty-four control cases he found the hyperplasia four times. Todyo arrived, as Erdheim had done, at the conclusion that in osteomalacia parathyroid hyperplasia is a sequel probably of increased functional demands on the gland.

In all of nineteen cases of senile osteoporosis, Danisch<sup>60</sup> found focal parathyroid hyperplasia; in a control series of twenty-eight cases of the same age group, he found this hyperplasia but occasionally. Enlargement of parathyroid glands has also been reported in cases of "hunger-osteopathy" (Schmorl<sup>61</sup>).

The association of osteitis fibrosa deformans (Paget's disease) with hyperplastic processes in the parathyroid glands has repeatedly been pointed out (e. g., Maresch<sup>62</sup>). This association is not a regular one,

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56. Erdheim: Denkschr. d. k. Akad. d. Wissenschaft. math.-naturw. Kl. **90**:363, 1914.

57. Ritter: Frankfurt. Ztschr. f. Path. **24**:137, 1920.

58. Erdheim: Sitzungsber. d. k. Akad. d. Wissenschaft. math.-naturw. Kl. **116**: 311, 1907.

59. Todyo: Frankfurt. Ztschr. f. Path. **10**:219, 1912.

60. Danisch: Klin. Wchnschr. **3**:1836, 1924.

61. Schmorl: München. med. Wchnschr. **67**:1277, 1920.

62. Maresch: Frankfurt. Ztschr. f. Path. **19**:159, 1916.

as cases of osteitis fibrosa deformans without any macroscopic or microscopic changes of the parathyroid glands have been observed (Hartwich<sup>63</sup>). The frequent but not regular coincidence of bone diseases with parathyroid hyperplasia serves to strengthen Erdheim's contention that the latter is not the primary cause of the former.

#### SUMMARY

Early experimental work furnished proof of a causal relationship between total parathyroidectomy and tetany and led to the establishment of the symptom complex known as tetania parathyreopriva. Subtotal parathyroidectomy was found to be followed by a state of latent tetany. It has been conclusively shown that tetania parathyreopriva is dependent on lowering of the calcium ion concentration in the serum and, probably, in the central nervous system. When, subsequent to parathyroidectomy, certain protein split products, the guanidines, were found to appear in increased quantity in the urine, some investigators were led to suspect a causal relationship between a derangement of the intermediate protein metabolism and tetany.

This view received support by reason of the similarity in symptoms existing between experimental guanidine poisoning and tetania parathyreopriva. It was thought that an increased effect of guanidines, through the resulting alkalosis, would lead to decrease of the calcium ion concentration. The question remains unanswered whether parathyroidectomy affects the calcium metabolism of the organism directly, or indirectly through an effect either on the intermediate protein metabolism or on the level of inorganic phosphorus in the serum.

Parathyroid function was found to be of importance in regard to the formation, the maintenance and the repair of bone as well as in regard to the formation of teeth. Incomplete calcification of regenerating teeth, incomplete, delayed calcification of the callus of fractured bones and decreased calcium storage of bones ensue from parathyroidectomy. The presence of an excessive amount of the specific hormone of the parathyroid glands in the blood, on the other hand, may also lead to decrease of the calcium content of the bones. In this case, the hypercalcemia resulting from the excessive parathormone content of the serum appears to be induced at the expense of the calcium of the bone.

Recent experimental work has clearly indicated that the effect of the parathyroid hormone depends on the total intake of calcium as well as on the supply of vitamin D. This interdependence of three factors influencing the calcium metabolism appears to be of considerable significance in regard to the pathogenesis of generalized osteitis fibrosa

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63. Hartwich: Virchows Arch. f. path. Anat. 236:61, 1922.



cystica, osteomalacia, osteoporosis and related bone diseases. These bone diseases are frequently associated with hyperplasia of the parathyroid glands. However, they occur in a good many instances without parathyroid hyperplasia, and again, the latter occurs without the bone diseases under consideration.

Such facts render it unlikely that hyperparathyroidism is the sole cause of osteitis fibrosa and the related bone disorders. On the contrary, they suggest that in the pathogenesis of these diseases hyperparathyroidism is not the primary etiologic factor. In apparent contrast to this inference stands the experimental reproduction of osteitis fibrosa cystica through repeated injections of parathormone. When one considers that disturbance of either the intake of calcium or of the supply of vitamin D may secondarily lead to hyperparathyroidism, it becomes possible to reconcile the anatomic and the experimental data available.

A primary metabolic disorder, concerning the intake of calcium or the supply of vitamin D, or both, may lead to a disturbance of the calcium storage of the bones. Simultaneously it may lead to a hyperactivity of the parathyroid glands. The latter, in turn, may result in lesions characteristic of osteitis fibrosa cystica, depending, however, again on the intake of calcium and the supply of vitamin D. At present, it remains an open question whether in the pathogenesis of osteitis fibrosa cystica hyperparathyroidism is an indispensable etiologic factor—as suggested by experimental results—or a secondary aggravating phenomenon.

## Notes and News

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**University News, Promotions, Resignations, etc.**—James Ewing, formerly professor of pathology at Cornell University Medical College, has been appointed director in full charge of the Memorial Hospital for the Treatment of Cancer and Allied Diseases. Recently Dr. Ewing was awarded the honorary degree of Sc.D. by the University of Rochester.

George Washington University has conferred the honorary degree of doctor of science on F. P. Gay, professor of bacteriology in Columbia University.

Frank L. Apperly, recently of the department of pathology in the University of Melbourne, has been appointed professor of pathology in the Medical College of Virginia at Richmond.

George W. Bachman has been appointed director of the School of Tropical Medicine of Columbia University, San Juan, Porto Rico.

Frank B. Mallory, pathologist to the Boston City Hospital, has been awarded the honorary degree of doctor of science by Boston University.

The doctorate of science was conferred recently by Colgate University on S. A. Petroff, director of the laboratory of Trudeau Sanitarium.

**Society News.**—The Ninth International Congress of the History of Medicine will meet in Bucharest on Sept. 10 to 18, 1932.

The Society for Experimental Biology and Medicine recently elected A. R. Dochez, president; E. L. Opie, vice president; A. J. Goldforb, secretary-treasurer.

At its last annual meeting in New Orleans, the American Society of Clinical Pathologists gave the Ward Burdick award to Benjamin S. Kline, Cleveland, Ohio, for work on the serological diagnosis of syphilis. R. R. Kracke was awarded the gold medal for his demonstration of agranulocytic angina in the scientific exhibit of the society. A. J. Foord is the president-elect.

**Tumor Clinic in the Edward Hines Veterans' Hospital.**—Approximately 200 beds are now devoted to cancer patients. Two and one-half grams of radium are available. In 1931 more than a thousand patients with neoplasms were under treatment. It is estimated that among 4,500,000 veterans neoplasms will develop in approximately 9,000 in 1932; further, that in 1942, when the number of veterans will be about 4,000,000, about 17,000 will have tumors.

**Manson Medal.**—According to *Science*, the Manson medal for tropical medical research, given triennially by the Royal Society of Tropical Medicine and Hygiene, has this year been awarded to Theobald Smith, who recently retired as director of the department of animal pathology of the Rockefeller Institute for Medical Research. The previous recipients of the Manson medal have been David Bruce (1923), Ettore Machiavava (1926) and Ronald Ross (1929).

**Trudeau Medal.**—"For outstanding service to the tuberculosis movement," the National Tuberculosis Association has awarded the Trudeau Medal to Esmond R. Long, until recently professor of pathology in the University of Chicago and now professor of pathology in, and director of the laboratories of, the Phipps Institute of the University of Pennsylvania.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE INFLUENCE OF FEEDING OF THYROID GLAND ON TISSUE RESPIRATION. E. ROBLES, Frankfurt. *Ztschr. f. Path.* **41**:193, 1931.

A series of white mice were fed with dried thyroid tissue and dried portions of the anterior lobe of the hypophysis. Over a period of from five to thirty days, from 0.09 to 0.1 Gm. of the preparation was given by mouth in addition to a diet containing bread, milk and oatmeal. The metabolism of the kidney, liver, lungs and spleen was determined according to the Warburg method. The determination was done twenty-four hours after the last feeding, so as to rule out digestion as a possible factor in explaining increased metabolism. Beginning with the fifteenth day after the introduction of the feeding, there was a distinct increase in tissue respiration. Because of the fact that thyroxine alone does not lead to an increase of tissue respiration, the author concludes that other important hormones were present in the material used in addition to thyroxine.

O. SAPHIR.

THE EXPERIMENTAL PRODUCTION OF AMYLOID BY MEANS OF IMPLANTATION OF ORGANS. J. CRUZ, Frankfurt. *Ztschr. f. Path.* **41**:250, 1931.

In a number of mice in the abdominal cavities of which parts of various organs of other mice were implanted, amyloid was demonstrated. It was found mainly in the spleen, but also in the kidneys, liver, lungs and heart. If parts of organs taken from guinea-pigs were implanted in mice, amyloid was found much less frequently. The author concludes that the absorption of homologous protein leads to a formation of amyloid more readily than the absorption of foreign protein.

O. SAPHIR.

LOCALIZATION OF LIPOID DEPOSITS. K. LÖWENTHAL, Frankfurt. *Ztschr. f. path.* **41**:374, 1931.

In mice, after repeated intraperitoneal injections of cholesterol in oil (a 3 per cent solution of cholesterol in peanut oil) fat emboli were found in the lungs and other organs, in addition to lipoid deposits in the endothelial cells of the blood vessels. Repeated intraperitoneal injections of neutral fats, however, led to the formation of fat emboli, but did not produce intracellular deposits of fats. Also, after subcutaneous injection of cholesterol in oil, deposits of lipoid could be demonstrated.

O. SAPHIR.

THE RESORPTION AND EXCRETION OF LIPOIDS AFTER A SINGLE ADMINISTRATION. M. LEFFKOWITZ, Frankfurt. *Ztschr. f. Path.* **41**:386, 1931.

In 55 mice, cholesterol in oil (5 per cent solution of cholesterol in peanut oil) was given either by mouth once or by one intraperitoneal injection. When such a lipoid was given per mouth, it reached the liver, was kept there, and was not found in the lungs or kidneys. When the cholesterol was injected intraperitoneally, it was brought by the thoracic duct into the lungs and was also found occasionally in the kidneys. In castrated mice, lipoids might also be present in the kidneys, following the feeding with cholesterol.

O. SAPHIR.

AUTOTRANSPLANTATION OF SPLENIC TISSUE. W. PUTSCHAR, *Verhandl. d. deutsch. path. Gesellsch.* **26:259**, 1931.

In twenty-four rats, the spleen was removed and reimplanted in three or four small pieces; in seventeen animals, these pieces of splenic tissue were placed in the peritoneal cavity; in seven, in the subcutaneous tissue of the abdominal wall. Twelve rats survived this operation for from four to five months. Only in two animals was the transplantation unsuccessful. Histologic examination of the grafts revealed living, functioning tissue. In half of the surviving cases, typical malpighian bodies were found in the transplanted pieces.

C. ALEXANDER HELLWIG.

THE RELATIONSHIP BETWEEN NODULES OF THE THYROID GLAND AND PREGNANCY. H. SCHLEUSSING, *Verhandl. d. deutsch. path. Gesellsch.* **26:304**, 1931.

The examinations were made in Düsseldorf, in the lower Rhine Valley, where goiter is not endemic. The incidence of nodules in thyroid glands of normal size was determined in 276 women more than 20 years old. The incidence and number of nodules increased with the number of childbirths. Of women without children, 25 per cent had nodules; of those with one childbirth, 40.4 per cent. The rate of incidence increased to 100 per cent in women with more than ten childbirths. In the latter group, all had more than one nodule in the thyroid gland. In pregnancy, the activity of the thyroid gland is increased; the resulting diffuse hyperplasia favors the development and growth of nodules.

C. ALEXANDER HELLWIG.

INTRACRANIAL PRESSURE IN ITS RELATION TO THE HYPOPHYSIS AND CYSTIC OVARIES. J. KRAUS, *Verhandl. d. deutsch. path. Gesellsch.* **26:308**, 1931.

In 45 of 50 patients with increased intracranial pressure due to a chronic process, the weight of the hypophysis was greater than normal. Microscopically, cellular hypertrophy and hyperplasia were found, especially of the principal cells in the anterior lobe. Only those showed hyperplasia in which the infundibulum and the floor of the third ventricle were intact. The cause of this enlargement is entirely unknown. In patients with metastases of malignant tumors in the brain and with chronic abscesses of the brain the weight of the hypophysis was not increased. In 83 per cent of the women of the series there was a small cystic degeneration of the ovaries. Since in several men and women with tumors of the brain causing intracranial pressure, the pituitary hormone was found increased in the urine, and since the injection of the hormone into animals causes cystic degeneration of the ovaries, there seems to be a relationship between the described changes in the hypophysis and the cystic degeneration of the ovaries.

C. ALEXANDER HELLWIG.

THE EFFECT OF SODIUM HEXOSEDIPHOSPHATE ON DIABETIC AND NORMAL ANIMALS. W. S. ILYIN and J. T. KUSNETZOW, *Ztschr. f. d. ges. exper. Med.* **79:635**, 1931.

The injection of sodium hexosediphosphate into a depancreatized animal causes a sharp and moderately lasting drop in the blood sugar, often to normal, and a rise in the inorganic phosphates of the blood. The level of the blood sugar in a normal animal is not affected by the injection of this substance. In certain experiments on diabetic animals with the sodium hexosediphosphate there occurred a reaction that was controlled by the use of ether; the drop in blood sugar, however, remained constant. Synthetic sodium glycerophosphate, injected subcutaneously, acts in the same way in diabetic as in normal animals in splitting off inorganic phosphate. Large doses of sodium glycerophosphate are toxic. Injection of the latter substance not only in small but also in large doses has no depressing effect on the

level of the blood sugar, even though the phosphate content of the blood rises. More exact knowledge of the part of phosphate, as well as of the complex esters of phosphoric acid, awaits further investigation.

FROM THE AUTHORS' SUMMARY.

STUDIES ON THE THYROID GLAND. H. OKKELS, M. KROGH and A. LINDBERG, *Acta path. et microbiol. Scandinav.* 9:1, 21, 37, 1932.

*Histology and Cytology of the Normal and Abnormal Human Thyroid Gland (H. Okkels).*—The mitochondria appear to show some relation to the production of colloid, and as a rule their number is increased in cases of exophthalmic goiter treated with iodine. The Golgi apparatus is distinctly hypertrophic in exophthalmic goiter, but its position in the cell has nothing to do with the direction of the secretion to either side, into the alveolus or into the capillary. There is no cytologic or histologic reason to assume that in exophthalmic goiter the thyrotoxicosis is due to anything else than an excessive hypersecretion into the blood stream, preventing the accumulation of colloid. Preoperative treatment with iodine, while for a time reducing the toxic symptoms and inducing an accumulation of colloid, does not influence the marked hypertrophy of the Golgi apparatus, and the cellular hyperactivity probably keeps on unabated. It is logical, therefore, to assume that the factor inducing this hypersecretion must be some extrathyroid principle, and if so, it must be a stimulant of nervous or humoral character.

*Physiologic Activity of Iodine in Thyroxine and in the Normal and Pathologic Thyroid Gland (M. Krogh and A. Lindberg).*—The biologic activity of thyroxine, dried thyroid gland of the guinea-pig and resected human thyroid gland on the metabolism of full grown male guinea-pigs has been compared by feeding doses corresponding, in each case, to 4.5 mg. of iodine per square millimeter of surface every other day for two weeks and measuring daily the resting metabolism at from 31 to 32 C. The activity of thyroxine is about two thirds that of dried thyroid gland of the guinea-pig. The iodine content of human thyroid gland varies greatly; in simple goiters, it is between 0.04 and 0.4 per cent; in exophthalmic goiters that have not been treated with iodine, between 0.03 and 0.07 per cent, and in iodine-treated exophthalmic goiters, between 0.03 and 0.4 per cent. The dried glands from simple goiters and from exophthalmic goiters successfully treated with iodine brought about an increase in metabolism of nearly the same magnitude as that caused by the normal guinea-pig's thyroid gland. Glands from nontreated exophthalmic goiters and from iodine-treated ones in which the treatment was ineffective both clinically and histologically showed a biologic activity per milligram of iodine markedly below the normal. The most probable explanation appears to be that in thyroid glands that are unable to store the hormone, giving it off to the blood as rapidly as it is formed, the intermediate products between inorganic iodine and the final hormone may make up a significant fraction of the total iodine, which probably is not the case in normal glands or in colloidal goiters.

*Experimental Hyperactivity of the Thyroid Gland (M. Krogh, A. Lindberg and H. Okkels).*—Extracts from the anterior lobe of the pituitary gland injected subcutaneously into young or full-grown guinea-pigs induce hyperactivity of the thyroid gland. As reliable criteria of hyperactivity of the thyroid gland after injection of the aforementioned extracts we found the following phenomena: 1. Considerable increase of the standard metabolism and pronounced hypertrophy of the Golgi apparatus in the thyroid gland cells, generally parallel with the increase in the metabolism. These experiments therefore establish the fact that the Golgi apparatus is an indicator of specific cellular activity. 2. Marked proliferative changes in the thyroid gland, with hypertrophy of the gland cells, increased vascularization and marked diminution of the colloid content. In pronounced cases, the microscopic changes in the gland are of such a nature and degree that they cannot be distinguished from those of human exophthalmic goiter. Peroral administration of the alkaline extract showed no effect. Subcutaneous injection of sex

hormone preparations of the anterior lobe of the hypophysis gave perhaps a slight decrease in the standard metabolism, but no structural changes in the thyroid gland. Peroral administration of large doses of dried thyroid gland or subcutaneous injection of thyroxine solution daily for up to one week produced a considerable increase in the metabolism, but no change in the thyroid gland. Peroral administration of dried thyroid gland in full-grown guinea-pigs for a period of eight months produced an increased accumulation of colloid in the alveoli and a slight degree of atrophy of the lining cells in the thyroid gland.

#### AUTHORS' SUMMARIES.

MAMMALIAN PARABIOSIS, WITH PARTICULAR REFERENCE TO THE SEX GLANDS AND THE HYPOPHYSIS. E. MÖLLER-CHRISTENSEN, *Acta path. et microbiol. Scandinav.* 9:55, 1932.

This is a general review of experimental mammalian parabiosis, with bibliography.

### Pathologic Anatomy

HISTOLOGIC STUDIES OF THE BRAIN IN CASES OF FATAL INJURY TO THE HEAD. C. W. RAND and C. B. COURVILLE, *Arch. Surg.* 23:357, 1931.

This study was directed to determining the changes occurring in the choroid plexus and the ependymal lining of the ventricles in fatal cases of traumatic injury to the head. Sixty-one brains in such cases were studied, along with ten normal brains as controls. Following trauma, definite changes occur in the choroid plexus. The free epithelial margin becomes irregular in outline, and the cells increase in height. Occasionally, the free epithelial margin is ragged and torn, and free fragments are found in the ventricular space. Vacuolization of the epithelial cells is prominent. Granulation of the cytoplasm is present and varies in amount, being more or less proportionate to the size and swelling of the cells. The nuclei become swollen and vesicular; occasionally, they are ruptured. Sometimes the nuclei shrink, and the chromatin becomes condensed. Autochthonous pigment is occasionally increased and is usually distributed in the periphery of the vacuoles. This pigment does not stain for iron. The basement membrane is sometimes torn, and fluid collects between it and the cells. The connective tissue stroma becomes prominent by virtue of edema, and this edema probably precedes the vacuolization in the cells. The changes in the ependymal epithelium are similar, but are rather less marked than those in the choroid. Subependymal edema is rather prominent. Increased vacuolization of the epithelium of the choroid and of the ependyma suggests that these cells actively secrete fluid, and it seems logical to conclude that following injury to the brain their activity is increased. This would account for the increased cerebrospinal fluid found in injuries to the brain. In those cases in which hypertonic dextrose was given intravenously shortly after the injury, the changes in the choroid plexus and the ependyma were less marked. In general, it may be stated that the changes noted in these fatal cases resemble experimental "water brain" of animals produced by intravenous injection of hypotonic solution.

N. ENZER.

THE TYPICAL POSITION OF THE MYOCARDIAL SCARS FOLLOWING CORONARY OBSTRUCTION. W. G. MACCALLUM and J. S. TAYLOR, *Bull. Johns Hopkins Hosp.* 49:356, 1931.

Three typical photographs from a large series are reproduced to show the contrast between obstruction of the anterior descending branch of the left coronary artery, obstruction of its circumflex branch and obstruction of the right coronary artery. Obstruction of the anterior descending branch usually results in scarring of the more anterior part of the interventricular septum, sometimes even extending though to the right ventricle, and of the apical and more anterior part of the wall of the left ventricle. Obstruction of the left circumflex branch

results in scarring and thinning of the wall of the left ventricle in its more median portion between the area of the left anterior descending branch and that of the right coronary. Obstruction of the right coronary artery, especially of the distal portion, produces dilatation and a scar that begins in the midline of the interventricular septum and extends backward behind the papillary muscles of the mitral valve to curve around on the most posterior part of the wall of the left ventricle. Scarring of the wall of the right ventricle is rare, and this may be due to the more direct course of the branches of this artery that supply it. If these anatomic changes are kept in mind, one can tell, from the position of the scarred area, which coronary artery is obstructed.

LUKE HUNT.

LESIONS IN THE LATERAL HORNS OF THE SPINAL CORD IN ACRODYNIA, PELLAGRA AND PERNICIOUS ANEMIA. S. T. ORTON and L. BENDER, *Bull. Neurol. Inst., New York* 1:506, 1931.

The lesions of the central nervous system in one case of acrodynia, one case of pellagra and five cases of pernicious anemia are described. In all the cases we have found severe lesions in the lateral horns of the lumbar and thoracic levels of the spinal cord and analogous areas at other levels. These lesions are all of a chronic type, characterized by loss of nerve cells and nerve fibers and by fibrous glial replacement. Since the lateral horn region contains the cell bodies of the effector neurons that serve to connect the spinal cord with the sympathetic nervous system, lesions in this locus are held to be in suggestive relation to the disturbances of vasomotor and splanchnic control which are common to these diseases.

AUTHORS' SUMMARY.

THE RELATIONSHIP OF THE SUBARACHNOID AND PERINEURAL SPACES. C. G. DYKE and E. M. DEERY, *Bull. Neurol. Inst., New York* 1:593, 1931.

A woman received an intraspinal injection of iodized poppy seed oil 40 per cent, which on roentgenologic examination was found scattered throughout the subarachnoid space from the fifth dorsal to the fourth lumbar vertebrae. At the sixth, seventh and tenth dorsal segments the iodized oil could be seen emerging from the intervertebral foramina; some was seen far out from the boundaries of the vertebral canal, and in the lumbar region rather large collections of iodized oil were seen outside the canal boundaries. "The distribution closely resembled the arrangement of the lumbar sacral plexus." In short, the iodized oil traveled from the subarachnoid space along the perineural spaces of the lumbosacral nerves; hence these spaces may be avenues of escape for arachnoid fluid.

GEORGE B. HASSIN.

ABSENCE OF THE CERVICAL SPINE (KLIPPLE-FEIL SYNDROME). G. I. BAUMAN, *J. A. M. A.* 98:129, 1932.

The Klippel-Feil syndrome is a numerical variation in the cervical vertebrae, with more or less complete fusion into one mass accompanied in some cases with spina bifida or other anomalies. Only thirty cases have been reported, mostly in the French literature. Six cases of this anomaly are reported by Bauman. In all of the cases there was marked diminution in the number of cervical vertebrae, and those remaining were fused into one mass. Some of the cases presented lesions of nerves, and the question has been raised as to whether the original lesion is in the nervous or in the osseous system. One symptom present in four of the cases was an inability to dissociate the movements of the two hands. Any movement performed by the right hand was copied more or less accurately by the left. The phenomenon has been designated the mirror movement. The classic symptoms as observed by Klippel and Feil are absence or shortening of the neck, lowering of the hair line on the nape of the neck and limitation of motion. Other symptoms occurring in a certain percentage of cases are torticollis, mirror move-

ment, facial asymmetry, dorsal scoliosis, other deformities, difficulty in breathing or swallowing and shortness of breath. Changes in individual muscles have been noted. Muscle spasm or almost constant contraction of the cervical muscles has been observed and explained on the assumption that the muscles must take the place of the osseous system in supporting the head. The neck is so short that the thorax almost reaches the head, Klipple and Feil referring to this as the cervical thorax. The deformity remains stationary. It is disguised with difficulty and unfortunately does not become less conspicuous as the patient develops. The mirror movement, when present, become less noticeable, but does not disappear. The deformity does not interfere with longevity. No treatment is of benefit. Operations should be avoided by the making of a correct diagnosis.

LUKE W. HUNT.

RUPTURE OF THE CORONARY ARTERY. C. B. BAMFORD, Brit. M. J. 1:842, 1931.

Two cases are reported. In one, which concerned a patient with dementia praecox, aged 64 years, the features of interest were the presence of multiple aneurysms of the coronary arteries, situated in an unusual position—namely, toward the terminal parts of the artery rather than near the place of origin; the rupture of one of these aneurysms into the pericardium; the condition of the aorta, suggesting a probable syphilitic origin of the aneurysms. In the second case, which concerned a patient with dementia, aged 69 years, the rupture of the coronary artery was the end-result of advanced arterial degeneration, associated with chronic renal disease and cardiac hypertrophy.

SACCULATED ANEURYSM OF THE MIDDLE CEREBRAL ARTERY. W. H. CHASE, J. Path. & Bact. 35:19, 1932.

A large, sacculated, ruptured aneurysm has been described on the right middle cerebral artery at the position of its cortical branches, and lying entirely within the right cerebral hemisphere. Histologic and anatomic study of the intracranial vessels suggests that the aneurysm developed as the result of a congenital defect in the wall of the vessel at a point of branching.

AUTHOR'S SUMMARY.

POLYPI COLI. W. SUSMAN, J. Path. & Bact. 35:29, 1932.

Polypi coli were found in 6 per cent of 1,100 necropsies. No preference for any particular segment of the colon was noted. In about one half of the cases of carcinoma, coli polypi coexisted, and in about one third of this group there were malignant polypi. Of the 66 cases showing polypi coli, 15, or about one-fourth, were also cases of carcinoma coli. Polypi coli occurred most commonly in males, and in persons in later adult life. Only 7 per cent of those affected were under 40 years.

AUTHOR'S SUMMARY.

CAPILLARY HAEMANGIOMA OF THE SPINAL CORD ASSOCIATED WITH SYRINGOMYELIA. D. S. RUSSELL, J. Path. & Bact. 35:103, 1932.

A case is described in which a capillary hemangioma (hemangioblastoma) of the cervical enlargement was associated with syringomyelia.

AUTHOR'S SUMMARY.

CARDIAC BERIBERI OF NURSLINGS. J. ALBERT, Hyg. soc. 60:1008, 1931.

Infantile beriberi is responsible for 28 per cent of deaths of infants under 1 year of age in the Philippines. Of the three forms (aphonic, pseudomeningitic and cardiac) the last is the most serious. At autopsy there are cardiac hypertrophy and dilatation (often only of the right side of the heart), diffuse congestion of



the viscera and anasarca. A degenerative neuritis of the vagus occurs with long duration of the disease. The malady is observed always in an infant between 1 and 3 months of age, nourished by a mother suffering from latent or "partially apparent" beriberi. The infant is generally well developed, often large, and considered in good health.

H. S. THATCHER.

LYMPHOGRANULOMATOSIS. TITU VASILIU et al., *Presse méd.* **40**:25, 1932.

This is the report of a session of the Société anatomique devoted to lymphogranulomatosis. The disease is generally accepted to be an infectious granuloma, but the tuberculous etiology is rejected. Bezançon reports that inoculations of tissue into animals have occasionally given rise to generalized adenopathies transmissible in series. The lymph nodes contained many eosinophils, but no organisms could be detected. Cornil in the same manner obtained reticular changes and microscopic abscesses in the spleen, which appeared after intervals of three, four and nine months. The wide variations in the histologic picture of Hodgkin's disease are emphasized, pseudoneoplastic, inflammatory and plasmocytic forms being described. All of the cell forms are traced to the hemohistioblast of Ferrata. Some authors have attempted to identify the Reed-Sternberg cell with the megalokaryocyte of the marrow on the basis of the morphology and the high platelet count. No essential distinction is believed to exist between the typical form of the disease and those showing extensions to the lungs and pleura, the bones and the skin. The spleen is involved in 95 per cent of the cases. Henschen calls attention to certain rare generalized hyperplasias of the reticulo-endothelial system that defy interpretation. They have been termed histiocytosis, endotheliosis and reticulosis.

A. F. DeGROAT.

THE GENESIS OF HYALINE BODIES IN TISSUES. F. PESCATORI, *Sperimentale, Arch. di biol.* **85**:25, 1931.

Study of hyaline bodies found in chronic inflammatory and in neoplastic tissues, especially of the gastric wall, suggests that they are almost always situated in the submucosa, and that their formation depends on atrophy of the epithelial or muscular tissues. Another possible explanation of their formation is an altered local metabolism that results in the accumulation of the products of metabolism in the tissues.

G. PATRASSI.

SKELETAL CONDITIONS IN CRANIORACHISCHISIS AND CRANIOSCHISIS. E. GIERKE, *Centralbl. f. allg. Path. u. path. Anat.* **53**:1, 1931.

Gierke studied the skeletal and particularly the cranial alterations in a cranio-rachitic and an anencephalic monster and compared them with normal fetuses 17 cm. and 46 cm. in length. He made paper models of the occipital bone, and by flattening or bending these he was able to approximate conditions found in the deformities in question. He is of the opinion that pressure of some sort is the cause of these arrests in development, but offers no suggestion as to the origin of such pressure.

GEORGE RUKSTINAT.

THE ETIOLOGY OF BLOOD CYSTS FOLLOWING HEMORRHAGIC PACHYMENINGITIS. L. HARANGHY, *Centralbl. f. allg. Path. u. path. Anat.* **53**:65, 1931.

In the body of a 25 year old male idiot, blind since the age of 1½ years, were found two huge cysts of the dura, partially calcified and communicating with one another. These cysts were separated in front for 8 cm., but communicated posteriorly through a 10 cm. aperture in the falx cerebri. The depth of the cyst on the right side was 5.5 cm. at the coronal suture, and 10 cm. at the place of greatest pouching in the parietal region. The left cyst was about 2 cm. smaller at these respective places. The cysts contained 1,100 cc. of orange-colored fluid in which

were suspended crystals of cholesterol. The brain was markedly flattened. The development of these cysts in the dura is ascribed to trauma at birth in which the falx cerebri was torn, providing a connection between the two sides of the skull. Hemorrhagic pachymeningitis then developed and later partial calcification of the wall of the cyst. Death was due to gangrene of the right lung and pleurisy.

GEORGE RUKSTINAT.

THE ETIOLOGY OF LIPOGRANULOMATOSIS. W. J. SCHLAPABERSKY, *Centralbl. f. allg. Path. u. path. Anat.* **53**:97, 1931.

The author reports investigations of a region of lipogranulomatosis, 20 cm. long, about and proximal to the right knee of a tabetic patient who died of phlegmon of the thighs and retroperitoneal tissues. Histologically, this region contained many cysts filled with clear fluid and having walls composed of concentric layers of hyalinized connective tissue. Some portions of the walls were calcified, and some of the cavities contained fat droplets and crystals, which were thought possibly to be fatty acids. About the right knee joint there were evidences of resorption of bone. The author comments on the classification of lipogranulomatosis suggested by Abrikosoff, and believes that the spontaneous type may possibly be neurogenic. The reason for this belief is furnished in the present report, in which definite evidence is set forth of an atrophy of bone and muscle occurring in tabes. Additional support for such a view is obtained from observations on spotted fever, which is cited as the most common precursor of spontaneous lipogranulomatosis. In this illness, lesions of the nervous system occur and may give rise to neuralgic pain. Another reason for believing neurogenic or trophic disturbances are intimately associated with lipogranulomatosis is the bilateral symmetrical extent of the lesions occasionally seen.

GEORGE RUKSTINAT.

STUDIES OF THE EXTRAHEPATIC BILE DUCTS. J. F. NUBOER, *Frankfurt. Ztschr. f. Path.* **41**:198 and 454, 1931.

In this extensive study, the author discusses the anatomic and histologic appearances of the extrahepatic bile ducts under normal and pathologic conditions. The article is not suitable for an abstract. For information, the original article must be consulted.

O. SAPHIR.

CONGENITAL LEUKEMIA. W. BÜNGELER, *Frankfurt. Ztschr. f. Path.* **41**:257, 1931.

Leukemia is reported in a stillborn infant of about seven months' gestation, measuring 39 cm. in length and weighing 1,620 Gm. The liver was enlarged, weighing 125 Gm.; its architecture was completely obscured. Histologically there were large myelocytic infiltrations between the capillaries and the hepatic cells. The cells revealed eosinophilic and neutrophilic granules. Relatively few nucleated red cells were found. The spleen weighed 55 Gm. The follicles were not recognizable. Histologically, a marked infiltration was noted by cells similar to those found in the liver. The architecture of the lymph nodes was obscured. No follicles or germinal centers could be made out. There was a fine reticulum in the meshes of which many myeloid cells were observed. The bone marrow was outspokenly gray, with a few red dots; it showed few erythroblasts and erythrocytes, but many myeloblasts, and neutrophilic and eosinophilic myelocytes. Four fifths of the cells gave a positive oxydase reaction. No iron-containing pigment was noted. Many myeloid cells were found in the myocardium, lungs, kidneys, pancreas, gastro-intestinal tract and skin. At postmortem examination, smears taken from the blood of the vena cava and heart revealed 80 per cent myeloblasts and myelocytes. The author discusses the question of whether this disease is identical with the leukemia of the adult.

O. SAPHIR.

METASTATIC MEDULLARY NECROSIS OF THE KIDNEYS. W. SCHÖMER, Frankfurt. Ztschr. f. Path. **41**:265, 1931.

Three cases of metastatic renal medullary necrosis are described. In one, the necrosis was confined to the medullae of the kidneys; in two, the cortices were also involved. In all three cases, similar bacteria were found in the kidneys and in the spleen. The lesions in the kidneys apparently were secondary to ulcerative endocarditis in one case and to thrombophlebitis as the result of otitis media in a second case. In the third case, there was apparently bacteremia, the port of entry of which could not be detected. An attempt is made to explain the selective necrosis of the media by the peculiar blood supplies of the medulla. O. SAPHIR.

OSTEOGENESIS IMPERFECTA. W. HOF, Frankfurt. Ztschr. f. Path. **41**:306, 1931.

A case of osteogenesis imperfecta is reported in a 5 months old girl. At the time of birth, a fracture of the right forearm, marked bending of both shanks, genu extrorsum and tali pes varus were noted. The child died of bronchopneumonia. The autopsy revealed plump extremities, a large head, multiple fractures, thickenings of the seventh and eighth ribs and internal hemorrhagic pachymeningitis. Both tibiae were markedly curved. The large fontanel was wide open and the skull very thin, with small hemorrhages below the periosteum. The dura was adherent to the skull. An enlargement of the thyroid gland was noted. Histologically, there was marked increase of cartilage throughout the bones, with some calcification. There was only slight formation of bone in the region of the calcified cartilage, in spite of the fact that the many coherent osteoblasts were found close to the epiphysis. The cortical layer was only slight in amount; its lamellated arrangement was missing. The bone marrow was markedly hyperplastic. Cysts filled with homogeneous material were found in the bone marrow. The older fracture revealed much callus, which in part contained cartilage. The author discusses the etiology of this disease, but does not believe that the hyperplasia of the bone marrow was the primary cause. O. SAPHIR.

### Microbiology and Parasitology

BACILLUS MUCOSUS INFECTION OF THE NEW-BORN. MARK JAMPOLIS, KATHARINE M. HOWELL, JOSEPH K. CALVIN and M. L. LEVENTHAL, Am. J. Dis. Child. **43**:70, 1932.

An outbreak of infectious diarrhea developed in a nursery for the new-born. The symptoms were striking in severity, and mortality was high. Apparently the offending organism was *Bacillus mucosus*, the virulence of which may have been enhanced by symbiosis with anhemolytic streptococci. The primary and outstanding pathologic finding in the fatal cases was acute enteritis, the mucous membrane of the ileum being red, swollen, finely granular and covered with reddish-gray mucus. Microscopic examination showed the mucosa to be infiltrated with polymorphonuclear leukocytes and lymphocytes. A few shallow ulcers were found, and the lymphoid tissue was hypertrophied. Cultures from the throats and stools of three nursery maids revealed practically pure cultures of *B. mucosus*. When these nursery maids were relieved of their duties, the outbreak promptly subsided.

FROM THE AUTHORS' SUMMARY.

BACT. TULARENSE IN THE EASTERN WOOD TICK, *DERMACENTOR VARIABILIS*. R. G. GREEN, Am. J. Hyg. **14**:600, 1931.

*Bacterium tularense* has been isolated directly from the eastern wood tick, *Dermacentor variabilis*, by inoculation of a guinea-pig and subsequent culture. Over an area of 20 square miles in central Minnesota the percentage of wood tick

infection was found to be less than 0.1 of 1 per cent during the summer of 1930. The animal culture derived from ticks was of low virulence as indicated by the type of lesion produced and by failure consistently to produce fatal infections in rabbits.

AUTHOR'S SUMMARY.

OBSERVATIONS ON HOOKWORM DISEASES IN COSTA RICA BASED ON POST-MORTEM FINDINGS. LOUIS SCHAPIRO and E. G. NAUCK, *Am. J. Hyg.* **14**: 705, 1931.

The point to be emphasized is that the principal seat of the worms is the jejunum, in which are also found the lesions occasioned by the presence of the worms: more or less numerous punctiform extravasations of blood, and catarrhal inflammatory manifestations and edema of the mucosa. In the cases in which hookworm disease was the cause of death, we almost always found the jejunum and duodenum infested. In less intense infestations, frequently only the jejunum contained worms. The ileum, in all degrees of infestation, was most frequently found to be free from worms and only occasionally served as a site of the specimens recovered. According to our experience, the worms, even when they are loosened from the intestinal wall, very often remained alive and mobile for more than twelve hours after the death of the host. Our observations have shown that *Ankylostoma duodenale* is also more resistant in this respect and remains alive longer than *Necator americanus*. Extensive bloody infiltration of the mucosa and submucosa is rare, despite the fact that the mucus found on the surface of the mucosa (which is frequently sticky and abundant) may be markedly stained with blood. In none of the bodies dissected did we see signs of severe hemorrhage. The interesting question of the origin of hookworm anemia cannot be settled on the basis of pathologico-anatomic findings alone. It is, however, evident that the worms found at autopsy for the most part contain little or no blood in their intestinal tracts; and except for the punctiform mucosal hemorrhages (sometimes as large as lentils) and an occasional bloody injection of the intestinal contents, no marked hemorrhages are found.

FROM AUTHORS' SUMMARY.

THE BEHAVIOR OF RABBIT VIRUS III IN TISSUE CULTURE. TEODULO TOPACIO and ROSCOE R. HYDE, *Am. J. Hyg.* **15**:99, 1932.

Of seventy-six Maryland rabbits inoculated with virus III, 83 per cent were susceptible, as compared with 85 per cent reported by Rivers and Tillett in New York, and nearly 100 per cent reported by Miller and Andrewes in England. This refractory state was found in old animals. It is due apparently to an immunity established as a result of the infection. Although our experiments in cultivation in series have shown that the virus was still infective in a dilution of 1:300,000,000, this is not an absolute proof that the virus had actually multiplied. However, we believe that this occurred. The viability of the virus depends on the presence of living cells in the cultures. A minute anaerobic coccus or diplococcus was seen in normal rabbit testis which in cultures kills virus III. A slight bacterial contamination is tolerated by virus III, but a heavy one destroys it. The virus from tissue cultures is more virulent than that from the animal itself, as judged by the severity of the lesions. Passage of the culture virus from animal to animal apparently adapts it to the animal type. The fluid expressed from the plasma clot of the tissue cultures was infectious as shown by inoculation of animals. We are in agreement with Andrewes on this point. In cultures of rabbit testis, the virus attacks the interstitial cells and never the sex or primary cells as evidenced by the formation of inclusions in the former. All types of interstitial cells are susceptible to the formation of inclusions. Inclusions of virus III form regularly in cultures of normal rabbit testis in normal plasma regardless of whether the tissues are exposed to the virus for ten minutes or for one hour at room temperature. We agree with Andrewes on this point. The presence of inclusions in a tissue culture of virus III indicates that the virus is viable. This was tested by

inoculation of animals and by using such cultures to inoculate other cultures that subsequently developed nuclear inclusions. The cytogenesis of the intranuclear inclusions in tissue cultures involves a disturbance in the nucleoli in cooperation with the margination of the nucleoplasm, which results in a body surrounded by a clear zone, the halo. These stages were observed in coverslip cultures fixed in alcohol solution of mercuric chloride and stained with eosin-hematoxylin. Microscopic observations of tissue cultures showing inclusions failed to reveal indications that the inclusions are motile. Cells containing inclusions continue to multiply until overcome by the virus, which finally destroys them. Examination of the stained coverslip cultures in different stages of growth illustrates this beyond question. The basophilic granules embedded in the inclusions in touch preparations, and the fact that the inclusions become autolyzed in cultures along with other cells, would indicate the protein nature of the inclusion. Moreover, fat stains failed to color the inclusions either in fresh smears of infected testis or in tissue cultures. We failed to infect immune testicular tissue previously soaked in Tyrode's solution for one hour even by exposing such washed tissue to the virus for the same length of time. This is contrary to the results obtained by Andrewes. Tissue cultures of normal rabbit testis when brought in contact with the virus for one hour failed to show inclusions in the presence of immune plasma. This result is in disagreement with that obtained by Andrewes. Since immune plasma inhibits the action of virus III on cultures of normal rabbit testis, and since the immune testicular tissue cannot be infected even after washing with Tyrode's solution, it appears that the immunity resulting from virus III infection is of both cellular and humoral type.

AUTHORS' SUMMARY.

THE INFLUENCE OF DIET ON EXPERIMENTAL COCCIDIOSIS IN CHICKENS. ENA A. ALLEN, *Am. J. Hyg.* **15**:163, 1932.

Chickens infected with *Eimeria tesella* were maintained under practically identical conditions except for diet. Some were given diets high in protein and high in vitamins, while others were given diets low in protein and low in vitamins, and the oocyst production was determined. It was found that the oocyst production was lower during the first five days in the chickens on the high diets but that after this time the production was higher.

PAUL MERRELL.

THE LOCALIZATION OF *GIARDIA CANIS* AS AFFECTED BY DIET. H. TSUCHIYA, *Am. J. Hyg.* **15**:232, 1932.

This investigation was undertaken with the view of determining the localization of *Giardia canis* throughout the intestinal tracts of four young puppies experimentally infected with the organisms. The results showed that the duodenum and jejunum were the optimum habitats. Encystment occurred at the level where the bacterial flora commenced to be of complex type. From the distribution of *Giardia canis*, the intestinal tract may be divided into four apparently distinct zones, differing with respect to the biology of the flagellates present in their respective contents. These may tentatively be designated as the zone of division (the upper part of the duodenum), the zone of optimum localization (the lower part of the duodenum and the entire jejunum), the zone of minimum localization and initial encystment (the ileum) and the zone of encystment (the large intestine, especially the cecum).

FROM THE AUTHOR'S SUMMARY.

INFECTIOUS ORAL PAPILLOMATOSIS OF DOGS. W. A. DEMONBREUN and E. W. GOODPASTURE, *Am. J. Path.* **8**:43, 1932.

Infectious papillomas occurring in the mouths of dogs are described. The general histologic characteristics of the lesions are very similar to those of human warts. Basophilic intranuclear bodies, similar to the Lipschütz bodies

of human warts, occur in a few of the large wart cells of the older lesions. Their connection with the etiologic agent of the disease remains to be proved. Judged from our experiments practically all puppies are susceptible to the disease, but little is known regarding the proportion of older dogs that are susceptible. The average period of incubation in healthy puppies varies from thirty to thirty-three days, but may be as much as ten days longer in malnourished, sickly puppies. The lesions usually heal spontaneously. Regression in the experimental lesions occurs somewhat earlier than in the natural infection. Puppies that have recovered from the disease are immune to reinfection. We have not succeeded in inducing the disease in rabbits, rats, mice, guinea-pigs, kittens or monkeys. In puppies, the disease is easily transmitted in series by means of Berkefeld filtrates obtained from the lesions. The virus possesses a high degree of cellular specificity, apparently affecting only the mucous membranes of the mouth. The virus may be preserved for long periods in equal parts of glycerol and saline solution, or by drying the infectious tissue in vacuo while frozen. Subjection of the virus to a temperature of 58 C. for one hour renders it noninfectious. A temperature of 45 C. for a similar period does not appreciably impair its virulence.

AUTHORS' SUMMARY.

VACCINE VIRUS PNEUMONIA IN RABBITS. R. S. MUCKENFUSS, H. A. MCCORDOCK and J. S. HARTEY, *Am. J. Path.* **8**:63, 1932.

A characteristic form of pneumonia can be produced in rabbits by the introduction of vaccine virus into the lungs. The alveoli first contain coagulated albuminous fluid and fibrin, and later a cellular exudate composed principally of large mononuclear cells. Necrosis of the exudate and of the alveolar walls leads to hemorrhage and to the appearance of polymorphonuclear leukocytes. The perivascular lymphatics are distended with coagulated fluid. The walls of many of the larger blood vessels are edematous and often show diffuse infiltration of all the coats by polymorphonuclear leukocytes. Guarrieri bodies have been demonstrated in the epithelial cells of the bronchi in four animals.

AUTHORS' SUMMARY.

HISTOPLASMOSIS (DARLING) WITHOUT SPLENOMEGALY. R. M. CRUMRINE and JOHN F. KESSEL, *Am. J. Trop. Med.* **11**:435, 1931.

In a case of lymphadenitis, fungi were found in the abdominal lymph nodes, spleen, liver, intestine and lungs. The organisms encountered and the structural changes produced resemble more closely the findings reported in cases of Darling's histoplasmosis than those in any other condition known to the writers. It would thus appear that the present report marks the fifth case of histoplasmosis to be reported to medical science, and the second to be recorded in North America. Two differences between this and previous cases are apparent, however: The spleen was not enlarged at any time, whereas splenomegaly has been a constant characteristic in the other four cases. This case exhibited acute colitis, and had death not resulted so early it is possible that the spleen eventually would have become enlarged. In addition to the small intracellular phase of the organism reported by previous workers, a second or extracellular phase is recorded in which the organism is surrounded by a "halo" or capsule of considerable thickness. Whether this is a true capsule or a reaction product of the tissue is uncertain, but comparison of this structure with the capsules formed by yeastlike stages of other fungi leads to the conclusion that it probably is a mucinoid capsule, produced by the organism itself.

AUTHORS' SUMMARY.

STREPTOCOCCUS VARIANTS. C. D. GALLAGHER, J. Bact. **22**:363, 1931.

Of the eighteen matt strains of *Streptococcus hemolyticus* carried on infusion agar without blood for from 18 to 170 transplants, only two strains exhibited ability to give rise to glossy variants. One of these strains required 49, the other 75, transplants before showing any glossy colonies. These glossy derivatives, growing with difficulty in the presence of whole, unheated blood and unable to produce as potent a toxin as their matt forms, acquired the ability to change the color of chocolate agar from dark reddish brown to light greenish yellow-brown, a phenomenon not previously described. One of the glossy variants, when grown in 1 per cent normal rabbit serum broth for seven transplants, gave rise to a very rough or pebbly form, which was serologically identical with the glossy form, and which preserved its ability to change the color of the chocolate agar. In the study of daily throat cultures taken over a period of from two to six weeks from five patients with scarlet fever and three with erysipelas, all colonies of *S. hemolyticus* were of the matt type and no colonies of the glossy or pebbly forms were isolated.

AUTHOR'S SUMMARY.

THE LYSIS OF PNEUMOCOCCUS BY SAPONIN. S. J. KLEIN and F. M. STONE, J. Bact. **22**:387, 1931.

Pneumococci are not dissolved by saponin when tested in plain broth culture. Treatment of the bacteria with cholesterol renders them susceptible to complete and rapid lysis by saponin. The cholesterol exerts a direct action on the bacteria, independent of bacterial reproduction. The bacteria must be in contact with the cholesterol for a definite period of time prior to the addition of saponin in order to obtain lysis. Excess of saponin inhibits the sensitization by cholesterol. Conversely, excess of cholesterol inhibits lysis by saponin. Animal fluids, e. g., blood, or ascitic and pleural fluids, act similarly to cholesterol. Evidence is presented to show that the activity of these fluids is due to their cholesterol content. When cholesterol is esterified, it loses its affinity for saponin, and becomes incapable of sensitizing pneumococci to saponin. The theory is advanced that the lysis is due to a union of saponin with the cholesterol assimilated by the bacteria during sensitization. The parallelism between saponin bacteriolysis and saponin hemolysis is noted.

AUTHORS' SUMMARY.

INFECTIOUS LARYNGOTRACHEITIS OF CHICKENS. J. R. BEACH, J. Exper. Med. **54**:801 and 809, 1931.

The causative agent of infectious laryngotracheitis of chickens was found to be present in bacteriologically sterile tracheal exudate, spleens and livers of diseased fowls. The causative agent was present regularly in the tracheal exudate, in the spleens of about 60 per cent, and in the livers of about 30 per cent of chickens with active infectious laryngotracheitis. Suspensions of the spleen and liver were less effective in inducing the disease than those made from the tracheal exudate. This finding, with absence of pathologic changes in the spleens and livers, would seem to indicate that they are not actively involved, but that the causative agent is carried to them by way of the blood. The disease could, in our experience, be produced only in chickens. Domesticated ducks and several wild and free-flying species of birds, including sparrows, crows, starlings, doves and pigeons, were found to be refractory, and so, too, were rabbits, guinea-pigs, white rats and one pig that was tested.

Experiments have shown that tracheal exudate from two strains of laryngotracheitis of chickens from New Jersey and two from California when suspended in bouillon and passed through Berkefeld V filters will produce the disease. Two of six Berkefeld N filters allowed the etiologic agent to pass, whereas four did not. Attempts to produce the disease with Seitz filtrates were unsuccessful. These results demonstrate that laryngotracheitis is caused by a filtrable virus which because of its size or some other property does not pass readily through the finer

filters. It has been shown that the serums from fowls that have recovered from an infection with one of the New Jersey viruses will neutralize the same strain and also the one California strain tested. In order to demonstrate neutralization conclusively it was necessary to titrate samples of dried virus and in the tests to use approximately ten infecting doses. The virus dried over calcium chloride for ten days and then stored in the refrigerator for sixty days produced disease. Kept over calcium chloride for a month it was still active, and when dried by Swift's method it remained alive for five months.

AUTHOR'S SUMMARIES.

HISTOPATHOLOGY OF INFECTIOUS LARYNGOTRACHEITIS IN CHICKENS. O. SEIFRIED, J. Exper. Med. **54**:817, 1931.

The characteristic lesions of infectious laryngotracheitis are ordinarily restricted to the respiratory tract and are most pronounced in the larynx and trachea. Sometimes the eyelids are affected. A certain percentage of the cases are associated with bronchitis and peribronchitis, and pneumonic areas and hemorrhages in the lung, while involvement of the nasal passages, communicating sinuses and eyes seems to be dependent on the mode of infection and the course of the disease. The virus affects the epithelial cells primarily, but soon inflammation develops in the submucosa and underlying parts. Edema is often extremely pronounced in the submucosa. The destruction taking place at later stages is due to edema, cellular infiltration and hemorrhages, and in some instances to secondarily invading bacteria. Characteristic intranuclear inclusions in the epithelial cells of the trachea are present in many cases. They bear a close resemblance to the inclusions occurring in herpes, varicella, virus III of rabbits and submaxillary gland disease of guinea-pigs.

AUTHOR'S SUMMARY.

THE DERMAL PNEUMOCOCCIC LESION IN THE RABBIT. K. GOODNER, J. Exper. Med. **54**:847, 1931.

An attempt has been made to analyze the factors involved in the development and localization of the dermal pneumococcic lesion in the rabbit. The character and quantity of the edema fluid that forms during the early phases of the lesion are intimately concerned in its development and spread. The fluid contains an antithrombic substance, probably derived from the pneumococci, and delayed coagulation probably facilitates its movement through the tissue. The direction of spread in the skin is determined by gravity, and the fluid finally localizes in the more dependent regions. The distance that the pneumococcic lesion travels and the characteristics of the local tissue have much to do with the amount of fluid that accumulates. Studies are also reported of some factors that alter the rate of spread of the edema fluid. An acceleration in rate occurs when *Bacillus influenzae* is used as an associative infective agent with the pneumococcus.

AUTHOR'S SUMMARY.

THE HISTOLOGIC CHANGES AND THE FATE OF LIVING TUBERCLE BACILLI IN TUBERCULOUS RABBITS. M. B. LURIE, J. Exper. Med. **55**:31, 1932.

The mononuclears of the liver, splenic pulp and bone marrow destroy tubercle bacilli more readily than those of the lung, kidney or splenic corpuscle. The multiplication of tubercle bacilli in an organ and their accumulation within mononuclears are accompanied by active new formation of these cells by mitosis. When these mononuclears are transformed into mature epithelioid cells and when tubercles have reached their maximum development, the bacilli have undergone extensive destruction and are disappearing. Tubercle bacilli of moderate virulence (human type and BCG) are usually effectively destroyed within epithelioid cells of all organs. In the lung and kidneys, bovine bacilli persist within epithelioid cells, but in other organs they are usually destroyed. Tubercle bacilli are less effectively destroyed within epithelioid cells collected in the alveoli of the lung than in those



forming tubercles in the interstitial tissues. After multiplication of tubercle bacilli has ceased, regeneration of mononuclears by mitosis becomes less active, and now Langhans' giant cells may be formed from preexisting epithelioid cells. Lymphocytes and encapsulation of tubercles by granulation tissue do not cause destruction of tubercle bacilli. Immediately after infection, accumulation of the less virulent types of tubercle bacilli in the tissues does not cause caseation, and the more virulent bovine bacilli produce this change only in the lung. Later, caseation occurs in the presence of a small number of bacilli, and must be thought of as due, in part at least, to sensitization.

AUTHOR'S SUMMARY.

THE TRANSFORMATION OF R PNEUMOCOCCI INTO S FORMS BY THE USE OF PNEUMOCOCCUS EXTRACTS. J. L. ALLOWAY, J. Exper. Med. 55:91, 1932.

Avirulent R pneumococci derived from S forms of a specific type may be changed by growth in broth containing anti-R serum and a heated, filtered extract of S pneumococci of a different type into virulent S organisms identical in type with the bacteria extracted. This has been accomplished in the case of R strains derived from pneumococci of type II, with extracts prepared from S forms of types III and I. The constituents of the extracts supply an activating stimulus of a specific nature in that the R pneumococci acquire the capacity of elaborating the capsular material peculiar to the organisms extracted.

AUTHOR'S SUMMARY.

THE ACCUMULATION OF IRON IN TUBERCULOUS AREAS. V. MENKIN, J. Exper. Med. 55:101, 1932.

Repeated intravenous injections of ferric chloride are followed by an increase in the survival time of tuberculous rabbits. In the particular series of experiments reported, this increase amounted to about 78 per cent over the average survival time of control rabbits. Tuberculous animals given repeated injections of ferric chloride increased in weight during part of the period of these injections. The level reached markedly exceeded that attained by control rabbits. Both control and experimental animals died of generalized tuberculosis. At the time of death there was no indication of any differences in degree of pathologic involvement between the two groups of animals.

AUTHOR'S SUMMARY.

MULTIPLICATION OF THE VIRUS OF MEXICAN TYPHUS FEVER IN FLEAS. H. MOOSER and M. R. CASTANEDA, J. Exper. Med. 55:307, 1932.

The virus of Mexican typhus fever has been shown to multiply abundantly in the following species of fleas: *Xenopsylla cheopis*, *Ceratophyllus fasciatus*, *Leptopsylla musculi*, *Ctenocephalus canis*, *Ctenocephalus felis*. In all fleas, *Rickettsia prowazeki* was demonstrated within the epithelial cells of the stomach and within the malpighian tubules. Whereas in infected lice enormous numbers of these organisms are discharged from the disintegrating cells into the intestinal content, only few rickettsiae are found in the lumina of the fleas' intestines. They are held back by the peritrophic membrane, which covers the mucosa of the entire stomach. Rickettsiae seem to enter the lumen of the intestine almost exclusively by the route of the malpighian tubules. Observations were made that seem to indicate that the fleas recover from the infection, and that they are able to regenerate the partly destroyed intestinal mucosa. An explanation is given for the relative harmlessness of fleas as vectors of typhus.

AUTHORS' SUMMARY.

EPIDEMIC DISEASES AMONG WILD ANIMALS. CHARLES ELTON, *J. Hyg.* **31**:435, 1931.

Outbreaks of epidemic disease are common in populations of wild animals, including species little influenced by contact with the diseases of human beings or domestic animals. Such epidemics form one of the commonest factors responsible for fluctuations in numbers of wild mammals. An attempt is made to summarize the available published records of such epidemics, while certain unpublished records are contained in an appendix. Little is known of the causes of these epidemics except in the cases of plague and tularemia. The fluctuations in numbers of some wild mammal populations are sufficiently regular to make the forecasting of epidemics possible. This method is already applicable to wild mice. Mouse periodicities are discussed in detail, with special reference to epidemics and their causes. Development of the methods of forecasting epidemics will make possible the prediction of epidemics among many other wild mammals, and render intensive pathologic and epidemiologic studies more practicable than they have hitherto been.

AUTHOR'S SUMMARY.

THE EFFECT OF CONCENTRATION AND OF VARIOUS TISSUE CONSTITUENTS ON THE VIRULENCE OF THE POLIOMYELITIS VIRUS. CLAUS W. JUNGBLUT, *J. Immunol.* **22**:99, 1932.

Titration of poliomyelitic virus cord suspensions of varying percentages shows that there is a particular concentration with maximum virulence, below which the dilutions become progressively less infective, and above which virulence is likewise diminished. Berkefeld filtrates of virus cord suspensions are at times more virulent than corresponding unfiltered suspensions, although exceptions occur. Neither normal monkey cord nor normal monkey brain, when added in vitro to virus filtrates, has any appreciable effect on the virulence of the virus. The results with convalescent cord are too irregular to indicate clearly the presence of a neutralizing principle in the immune tissue. Normal monkey testicle added to virus filtrates in vitro frequently produces a conspicuous diminution of virulence of the virus in the supernatant fluid. The extent of this antagonistic effect varies with different monkey testicles. Normal rabbit testicle, under similar conditions, seems slightly to enhance the virulence of the virus.

AUTHOR'S SUMMARY.

MICROBIC DISSOCIATION IN THE BRUCELLA GROUP. M. S. MARSHALL and DOROTHY JARED, *J. Infect. Dis.* **49**:318, 1931.

By means of prolonged cultivation on agar containing specific antiserum and repeated selection, R types of most strains of *Brucella* are obtained. The R forms are relatively stable, and the S→R change appears to be continuous. Metabolic differences between S and R types are not clear. S types of various origins in guinea-pigs regularly produced pathologic changes, low agglutination titers and positive cultures. The corresponding R types induced few signs of infection. In three rabbits a hypopyon was produced by S cultures but not by R cultures.

EDNA DELVES.

CULTURES OF *TREPONEMA MICRODENTUM*. E. E. ECKER and L. A. WEED, *J. Infect. Dis.* **49**:355, 1931.

Contaminated cultures of *T. microdentium* were purified by centrifugation. Transfer of the surface layers of the centrifugated cultures to gelatinized human serum containing livers of guinea-pigs yielded pure strains.

AUTHORS' SUMMARY.

ISOLATION OF THE SPIROCHETE OF RAT-BITE FEVER FROM THE SALIVA OF RATS. L. BORBI, *Pathologica* **23**:120, 1931.

The saliva of rats contains a spirochetal form that is pathogenic for the guinea-pig and that is probably identical with the causal agent of rat-bite fever in man. The spirochetes of rat-bite fever and of spontaneous spirochetosis of rats are identical. Experimental infection is readily produced in guinea-pigs.

E. HAAM.

THE GROWTH OF BACTERIA IN DEAD TISSUES. G. TRUFFI, *Pathologica* **23**:205, 1931.

Dead tissues, even of immune animals, not only lose the antagonistic power of the development of the anthrax bacillus, but become, without the addition of any other nutritional material, a very favorable medium for the growth of the bacillus.

E. HAAM.

INGUINAL LYMPHOGRANULOMA. A. COHN and L. KLEEGER, *Dermat. Wchnschr.* **92**:580, 1931.

Intracerebral injections of 0.1 cc. of an emulsion of pus diluted 1:5 from a lymphogranulomatous lymph node were made in a *Cynomolgus* monkey. The animal died in sixteen days, after the appearance of disturbances in equilibrium, somnolence and refusal to eat. The hyperemic meninges were cut up fine, mixed with a few cubic centimeters of physiologic solution of sodium chloride and, together with the cerebrospinal fluid, sterilized at 60 C. for one and one-half hours on two successive days. A skin test, performed after the method of Frei, was made on five patients with inguinal lymphogranuloma and on five healthy controls, 0.1 cc. of the emulsion consisting of cerebrospinal fluid and meninges. A positive reaction in the form of a papule with an erythematous margin developed in the five patients, but not in the controls. It is concluded that the virus causing a cutaneous reaction in patients with inguinal lymphogranuloma is transmissible to monkeys, and that it is not inactivated for the cutaneous reaction by heating for three hours at 60 C.

LAWRENCE PARSONS.

AGRANULOCYTOSIS AND MYELOGENOUS LEUKEMIA IN TWO SISTERS INFECTED WITH THE SAME MICRO-ORGANISM. ERNST WOLFF, *Folia haemat.* **44**:38, 1931.

Two sisters died, one with signs typical of agranulocytosis, the other with those characteristic of aleukemic myelo-adenosis. The fact that in both instances *Streptococcus hemolyticus* was present in the blood and in the tissues led the author to the conclusion that agranulocytosis is not a disease *sui generis*, but is a reaction to sepsis. He quotes other writers who are of the same opinion.

B. M. FRIED.

THE TRANSMISSION OF INGUINAL LYMPHOGRANULOMA TO GUINEA-PIGS. K. MEYER, H. ROSENFELD and H. E. ANDERS, *Klin. Wchnschr.* **10**:1653, 1931.

The subcutaneous injection of exudate or crushed lymph gland tissues of patients with inguinal lymphogranuloma into guinea-pigs transmits the disease. Inoculations in ten cases were successful. The disease was transmitted from one guinea-pig to another, and in this way one virus strain was transplanted to the fifth, and another to the twelfth, generation. The virus passes through the Berkefeld filter and is markedly resistant to heat and the action of glycerin. The specific agent transmitted to the guinea-pig is not localized at the site inoculated but

spreads by way of the lymph channels and the blood. Systemic lesions occur in the liver and lungs. The diseased lymph nodes of the guinea-pigs are histologically the same as those of man.

AUTHORS' SUMMARY.

THE TRANSMISSION OF INGUINAL LYMPHOGRANULOMA TO RABBITS AND GUINEA-PIGS. H. FREUND and F. REISS, *Klin. Wchnschr.* **10**:1658, 1931.

In rabbits infected by the subdural inoculation of material from cases of inguinal lymphogranuloma, a characteristic meningeal encephalitis developed. Enlargement of the lymph nodes of the groins of rabbits was produced with material from man as well as with brain tissues from specifically diseased animals. These lymph glands had the characteristic histologic changes. Similar transmission experiments were successful in guinea-pigs. The infection can be transmitted from one animal species to another by inoculation.

ENDOCARDITIS DUE TO BACILLUS INFLUENZAE. H. FRANK, *München. med. Wchnschr.* **78**:1509, 1931.

The author reports chronic endocarditis caused by *B. influenzae*. His review discloses that in the twenty-one cases reported, none of the patients lived and only one became free from bacteria. The endocarditis caused by *B. influenzae* corresponds clinically to the endocarditis produced by *Streptococcus viridans*.

THE EFFECT OF HUMAN TUBERCLE BACILLI ON PIGEONS. M. A. KUSCHNARJEV, *Virchows Arch. f. path. Anat.* **276**:95, 1930.

The inoculation of human tubercle bacilli into pigeons resulted in the formation of a nonspecific granulation tissue in all the organs. The bacilli could be recultivated from the blood and organs of the infected pigeons, but guinea-pigs given injections of an emulsion of the organs remained healthy.

W. SAPHIR.

THE NATURE OF THE BACTERIOPHAGE. ERNST FRANKEL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:278, 1931.

Two bacteriophages (Flexner and Colon) were highly concentrated by adsorption with aluminum hydroxide and subsequent washing out with phosphate solutions. An attempt to achieve the same by centrifugating at a very high speed was not successful.

I. DAVIDSOHN.

RABIES VIRUS IN HUMAN SALIVA. H. PALAWANDOW and A. I. SEREBRENNAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:350, 1931.

From a study of five cases it appears that the saliva of patients affected with rabies contains the virus, and that from 2 to 3 cc. of it is necessary for intramuscular injection, preferably into guinea-pigs, which ought to be kept in a cool place and on a meager diet.

I. DAVIDSOHN.

THE OCCURRENCE OF UNDULANT FEVER IN SWEDEN. GUNNAR OLIN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:531, 1931.

The first case of undulant fever in Sweden was diagnosed in 1927. Since then about 100 cases have been reported annually. The infection is prevalent in cattle. An agglutinin titer up to 1:80 was found in the blood of persons living in an isolated section of Sweden, where the infection does not occur in cattle; such a titer was therefore set as the limit for nonspecific agglutinins. In a large number of serums, specific agglutinins were found in 0.56 per cent and complement-fixing antibodies in a similar number. They were all from persons who had never had symptoms or a history of undulant fever and who apparently went

through a latent infection. This explains why the disease is relatively more prevalent among the urban population; the immunity among the rural population is greater. Children are, as a rule, not affected. Women are affected twice as frequently as men.

I. DAVIDSOHN.

THE INFLUENCE OF THE BACTERIOPHAGE ON THE HEMOLYTIC ACTION OF TYPHOID BACILLI (PHENOMENON OF FRIEDBERGER-VALLEN). L. BIANCHI and C. CALLERIO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:155, 1931.

About 80 per cent of the strains of typhoid bacilli used produced slight hemolysis of the red blood cells of sheep, but not those of man or of rabbits. The addition of bacteriophage made nonhemolytic or only slightly hemolytic strains acquire marked hemolytic properties. In some cases bacteriophage was demonstrated in the cultures of spontaneously hemolytic strains.

I. DAVIDSOHN.

## Immunology

ANTISERUM IN EXPERIMENTAL POLIOMYELITIS. B. F. HOWITT, *J. Infect. Dis.* **50**:26, 47, 1932.

The prolonged immunization of two goats and a sheep with poliomyelitic virus over a period of years resulted in the development of antiviral substances that were capable in a certain number of instances, of protecting monkeys against infection, both when the substances were tested by the different methods of in vitro neutralization and when used therapeutically or prophylactically.

In monkeys in the preparalytic stage the percentage of recovery from experimental poliomyelitis was greater when serum from convalescent monkeys was given intramuscularly than when it was administered by the combined intrathecal and intravenous method. Paralysis, however, was rarely prevented, and with one exception recovery was accompanied by varying degrees of atrophy of the affected muscles. One hundred per cent mortality occurred if treatment was given after the onset of paralysis, while repeated injections of serum seemed no more effective than a single dose.

AUTHOR'S CONCLUSIONS.

ANTIBODY RESPONSE TO TYPHOID VACCINE. L. TUFT, E. M. YAGLE and S. ROGERS, *J. Infect. Dis.* **50**:98, 1932.

Comparison of the titers of agglutinins and of complement-fixing antibodies after intradermal, subcutaneous, intramuscular, intravenous and oral methods of administration of mixed typhoid vaccine reveals a uniformly better and more persistent response after local injection, particularly after intradermal injection. Such results may be explained on the basis of local stimulation of the formation of antibodies by the reticulo-endothelial cells, which are particularly abundant in the skin and subcutaneous tissues, or possibly by a slow absorption of the antigen. The antibody response after any of the methods showed definite dependence on the antigenic potency or the age of the vaccine—the older the vaccine, the less the response. The general observations of others as to time of appearance of the agglutinins and complement-fixing antibodies, the variability in titer and the tendency to early disappearance were corroborated in our studies. A complete lack of uniformity in the titers of agglutinins and complement-fixing antibodies was observed with many serums; an adequate explanation for this is not apparent. The antibody response after oral administration of the vaccine was completely negative, in spite of the use of different strains of vaccine and different groups of persons. From a therapeutic standpoint, the intradermal injection of small doses of a freshly prepared and antigenically potent mixed typhoid vaccine receives experimental justification and has in our hands proved satisfactory.

AUTHORS' SUMMARY.

THE FLOCCULATION REACTION WITH STAPHYLOCOCCAL TOXIN. F. M. BURNET, *J. Path. & Bact.* **34**:759, 1931.

A true toxin-antitoxin flocculation can be obtained with staphylococcal toxin and antitoxin. The point of optimal flocculation is constantly related to the neutral point as judged by hemolytic titrations, but is always in the zone of antitoxin excess, usually corresponding to a mixture of about 0.6 equivalent of toxin to 1 of antitoxin. The supernatant fluid from flocculation tests made with optimal proportions of toxin and antitoxin always contains free antitoxin. The washed toxin-antitoxin floccules are soluble in acetate solution at  $p_H$  3.3. From such a solution either toxin or antitoxin can be recovered by suitable methods. Carefully prepared anatoxin shows flocculations unaltered from those of the original toxin. The binding power of anatoxin under the conditions of the flocculation test is equal to that of the toxin of origin in contrast to the behavior in dilute solution, where it is half that of the toxin. Staphylococcal toxin is relatively highly resistant to heat. The effects of heating to 100 C. on hemolytic titer, binding power and flocculating power are described and compared.

AUTHOR'S SUMMARY.

ACTION OF ANTIVACCINIAL SERUM ON VACCINIA VIRUS. R. W. FAIRBROTHER, *J. Path. & Bact.* **35**:35, 1932.

Contact in vitro between immune serum and vaccinia virus plays an important rôle in determining the infectivity of the mixture on intracerebral inoculation. This is in marked contrast with the results obtained by intradermal inoculation.

AUTHOR'S SUMMARY.

SALMONELLA AGGLUTINATION AND RELATED PHENOMENA. P. B. WHITE, *J. Path. & Bact.* **35**:77, 1932.

In addition to the carbohydrate haptens characterizing the S and R forms of Salmonella, an alcohol-soluble protein, here termed Q, is concerned in the somatic serology of these organisms. Q is readily extracted by warm 95 to 97 per cent alcohol in the presence of hydrochloric acid. Q is a full antigen and stimulates the development of potent precipitating antibodies. Q occurs in all the serologic types and variant forms of Salmonella, and up to the present no evidence of interspecific or intervarietal differences has been obtained. Besides the ordinary S and R forms of Salmonella there exist rough races, derived from strains long cultured in the laboratory, which lack the S and R carbohydrate haptens, and in which no corresponding constituent has yet been demonstrated. These races, here named -forms, differ in agglutinative properties from ordinary R forms. S. forms do not appreciably agglutinate with anti-Q serums, though Q is certainly a constituent of the smooth surface. With anti-Q serums the -forms (and intermediate R forms) agglutinate much more vigorously than do ordinary R forms, which are very slowly clumped. Q fractions from coliform, dysentery and proteus bacilli have proved serologically similar to those obtained from salmonella bacilli, but no cross-reaction has been obtained with anti Q (salmonella) serums and material extracted from staphylococci and tubercle bacilli. The process of extracting Q, though it does not damage or interfere with the essential reactions of the carbohydrate haptens, causes a severe reduction, or almost complete loss, of somatic agglutinability. It is possible that this loss of somatic agglutinability is actually due to the removal of Q from the surface of the bacilli, in that this substance is precipitable by physiologic salt solution and has to a marked degree the power of clumping and carrying down bacteria in suspension; attempts to return lost agglutinability by adding Q to mixtures of antiserum and desensitized bacilli have been in part successful. An attempt is made to formulate a theory of somatic agglutination on this basis.

AUTHOR'S SUMMARY.

FRACTIONATION OF TYPHOID-IMMUNE RABBIT SERUMS. M. L. AHUJA, Indian J. M. Research **19**:601, 1931.

The euglobulin fraction of typhoid-immune rabbit serum is responsible for the floccular H and the granular O types of agglutination. The albumin and pseudoglobulin fractions are devoid of H and O agglutinins. The effect of inactivation for twenty minutes at 55 C. and aging at room temperature for from forty-eight to ninety-six hours was studied on the agglutination titer of twelve typhoid-convalescent human serums and three typhoid-immune rabbit serums. No marked difference in O and H agglutination titers was observed in either series.

AUTHOR'S SUMMARY.

AGGLUTINATION IN LEISHMANIASIS. R. ROW, Indian J. M. Research **19**:641, 1931.

The agglutination is a group reaction, and agglutinins are definitely demonstrable in all cases of kala-azar, in some more than in others. The agglutinins are usually not found in cutaneous leishmaniasis. The antibodies are most easily formed in the rabbit by injections of the products of *Leishmania*. The agglutination test is of scientific interest, but will not replace other tests for the clinical diagnosis of leishmaniasis, and is of no value in the differential diagnosis of the several members of *Leishmania*.

EDNA DELVES.

SERUM PROPHYLAXIS AND TREATMENT IN TETANUS. S. MUTERMILCH and E. SALAMON, Ann Inst. Pasteur **47**:277, 1931.

Intraspinal administration of tetanus antitoxin was much more effective than subcutaneous injection in protecting rabbits against toxin inoculated intramuscularly. This was true whether the serum was given before or after the toxin.

Neither toxin nor antitoxin could be demonstrated in the peripheral nerves of rabbits after intraspinal injection. Antitoxin inoculated into the brain was fixed by cerebral tissue and was not completely eliminated until after six days. Hence, it is believed that neutralization takes place in intimate contact with the central nervous system rather than in the peripheral nerves or in the cerebrospinal fluid when the serum is injected intrathecally. Rabbits responded more readily to vaccination with anatoxin when given intraspinally.

It is recommended that human tetanus be treated with a mixture of antitoxin and anatoxin injected intraspinally under chloroform anesthesia.

J. B. GUNNISON.

## Tumors

THE VITAL STAINING OF RABBIT CARCINOMA. R. C. TILGHMAN and F. C. LEE, Bull. Johns Hopkins Hosp. **49**:360, 1931.

In the first series of animals, trypan blue alone was injected, and it was found that the dye lodged primarily in the phagocytic wandering mononuclear cells, which were situated in great numbers in the stroma surrounding the tumor nodules. To a much less extent were these cells found within the nodules. Only occasionally did the cancer cells take up the dye, and in such instances the cells were situated near the periphery of the nodule. In the second series of animals, a course of trypan blue was given first, followed by a similar series of injections of carmine. By the use of these two contrasting dyes it was seen that the oldest areas of carcinomas stained predominantly blue and the youngest red, while a mixture of these two colors was seen in the regions of intermediate growth. The dye was in the phagocytic cells; some cells contained only the blue and others only the red, but the majority contained both dyes; never were cells seen in which a vacuole contained particles of each dye. The fact that some of these cells had

only the blue dye showed that they were incapable of taking up the red. In this respect, an instance is afforded in which a "blockade" of certain cells of the reticulo-endothelial system occurred, and an exception is thus furnished to the general belief that such cells cannot be blocked. The tremendous number of these cells, particularly in contrast to the relatively few lymphocytes, indicated a peculiar significance for them in cancer growth. Furthermore, their distribution in the area of cancer suggested that their ability to move about in the tissues had been exaggerated. Again, the young, or red-stained, phagocytic cells are always present at the areas of cancer necrosis, even if the surrounding tissue was composed of relatively old cancer cells as judged by the predominantly blue color of the macrophages. Whether present in the cancer cell itself or in the monocyte, apparently the dyes did not give any indication of retarding cancer growth.

AUTHORS' SUMMARY.

INCIDENCE OF CANCER IN TARRED AND SHELTERED MICE. P. ROUS and E. BOTSFORD, *J. Exper. Med.* **55**:247, 1932.

Mice sheltered for long periods from the intercurrent access of living entities out of the environment developed far cancer with the same frequency as controls exposed to an unusual extent. This happened although the food of the two groups differed profoundly in character.

AUTHORS' SUMMARY.

NUTRITIONAL REQUIREMENTS IN VITRO OF NORMAL AND MALIGNANT MOUSE EPITHELIUMS. L. SANTESSON, *J. Exper. Med.* **55**:281, 1932.

The cultivation in vitro of mouse tissues derived from normal organs, from eighty-six spontaneous epithelial tumors of the mammary gland, and from twenty-seven Ehrlich carcinomas has been undertaken, together with a study of the properties of the various cell types. The tissues liquefied fibrin from mouse and rat plasma more readily than fibrin from chicken plasma. Clots made of chicken plasma alone, if thoroughly washed, did not inhibit the migration of the cells. Normal and tumor tissues liquefied fibrin from the mouse, rat and chicken more actively than Ehrlich carcinoma did. Mouse epitheliums, both normal and malignant, showed greater activity than connective tissue cells from the same origin and were not overgrown by the latter. Mouse epithelium was more active in rat serum than in mouse or chicken serum, and, in embryonic juice from chickens, mice and rats. None of these fluids, however, supported cell proliferation indefinitely, except in the case of the Ehrlich carcinoma. These results indicate that mouse tissues possess nutritional requirements that are different from those of fibroblasts and epithelial cells of other animals. Nutritive mediums that suffice for prolonged cultivation of the normal and malignant tissues of the rat and the fowl, and also of the Ehrlich carcinoma, are not suitable for the cultivation of adult mouse epithelium derived from normal organs or from spontaneous tumors of the mammary gland. Rat serum supported the life of spontaneous tumors for a limited period of time only, whereas it enabled the Ehrlich carcinoma to proliferate indefinitely. Cells of normal organs and spontaneous tumors were not capable of invading normal tissues as Ehrlich carcinoma did.

AUTHOR'S CONCLUSIONS.

BRONCHOGENIC CARCINOMA. F. R. MENNE, M. BISAILLON and T. D. ROBERTSON, *Northwest Med.* **30**:155, 1931.

A study of sixteen cases of primary bronchogenic carcinoma with correlation of the pathologic and clinical findings is presented. Primary bronchogenic carcinomas are divisible into two distinctive groups, the hilar nodular and the diffuse necrotic. Further pathologic classification is superfluous and unnecessary. It is pointed out that the cell type does not offer an exact means of differentiating the various forms. The initial site may be found in any part of the bronchial lining



or the submucous glands, but is most frequent in the primary and secondary bronchi. Primary bronchogenic carcinoma probably never arises from the atrial lining cells. The symptoms, physical signs and roentgenologic findings can all be explained on the basis of the pathologic changes. In our opinion there is a definite increase in the incidence of primary bronchogenic carcinoma.

AUTHORS' SUMMARY.

ADSORPTION EXPERIMENTS WITH THE VIRUS OF ROUS SARCOMA. ANTOINETTE PIRIE, Brit. J. Exper. Path. **12**:373, 1931.

The adsorption of the virus of Rous sarcoma onto, and the elution of it from, alumina and kaolin is described. Such elutes are inactivated during incubation as rapidly as the crude filtrates, and are preserved by cyanic acid in the same way. The nitrogen of the infective dose of the elutes is of the order of  $10^{-3}$  mg.

AUTHOR'S SUMMARY.

EXPERIMENTS ON THE PRODUCTION OF TUMOURS ON THE SOMATIC MUTATION HYPOTHESIS. J. C. MOTTRAM, Brit. J. Exper. Path. **12**:378, 1931.

A sarcoma-like tumor appeared in a rat at the site of inoculation of a testicular emulsion which had been exposed to radiation in vitro. This was the only tumor arising in many experiments of a similar kind.

AUTHOR'S SUMMARY.

AN APPARATUS FOR THE PREPARATION UNDER STANDARD CONDITIONS OF A HIGHLY POTENT CARCINOGENIC AGENT IN MICE OF LOW TOXICITY. A. F. WATSON, Brit. J. Exper. Path. **12**:441, 1931.

An apparatus originally described by Hague and Wheeler (1929) for their work on the pyrolysis of the paraffins has been adapted for the preparation from purified turpentine of a carcinogenic agent under standard conditions of temperature, etc. The pyrolysis of purified turpentine in this apparatus at 850 C. yields a tar that, after steam distillation, exhibits high carcinogenicity combined with low toxicity when tested on mice. Charts showing the relative effectiveness of the tar prepared in this apparatus, which is simple in type and control, compared with two coal gas tars, are included.

AUTHOR'S SUMMARY.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Feb. 25, 1932*

PAUL KLEMPERER, *President, in the Chair*

SUBACUTE BACTERIAL ENDOCARDITIS SUPERIMPOSED ON RHEUMATIC AORTIC VALVULAR DISEASE WITH NO PREVIOUS INVOLVEMENT OF THE MITRAL VALVE. IRVIN GRAEF and CLARENCE DE LA CHAPELLE.

A white youth, 18 years of age, came to necropsy from the Third (New York University) Medical Division of Bellevue Hospital. The outstanding features of the past history were four attacks of rheumatic polyarthritis at 8, 14, 15 and 17 years, and frequent attacks of tonsillitis. Clinically, the picture presented was typical of subacute bacterial endocarditis superimposed on an old rheumatic condition of the heart.

At necropsy, the contour of the body and the distribution of the hair were those of a female. The skin was delicate in texture and practically hairless, there being a slight growth in the axillae and over the pubes. In the conjunctiva of the left eye there was a small petechial hemorrhage. There was moderate edema of the lower extremities. Clubbing of the fingers was present. A few grayish-pink remnants of the thymus were found scattered through the fatty tissue in the upper and anterior part of the mediastinum. Bilateral hydrothorax was present, as well as confluent lobular pneumonia of the right upper lobe.

The pericardial sac contained about 500 cc. of clear, yellowish fluid. At the site of the mouth of the coronary sinus there was a small network of fine endothelial bands, in the meshes of which was found a small, grayish-red, firmly attached thrombus. The left auricle was small. The posterior leaflet of the mitral valve showed a few small verrucous vegetations along the line of closure. The aortic leaflet was the seat of two large vegetations, one of which had ulcerated through the substance of the leaflet, causing a fenestration about 4 mm. in diameter. The valve substance elsewhere was slightly thickened, but no blood vessels were seen. The chordae tendineae of this leaflet were slightly thickened, and vegetations had grown down on their surface. The posterior leaflet of the mitral valve was thin, transparent and not vascularized. Its chordae were thin, delicate and not fused or shortened. The left ventricle was hypertrophied and dilated. The aortic valve was stenotic and insufficient. The commissures of all the cusps were firmly fused and adherent. The right anterior cusp showed a triangular-shaped vegetation on the ventricular surface, which was about 8 mm. in length and 4 mm. in width. It was yellowish white and soft. The left anterior cusp had two irregular vegetations on its ventricular surface, which were similar to the other vegetations. The posterior cusp showed no vegetations. The edges of all the cusps of the aortic valve were rolled, thickened and shortened, and the sinuses of Valsalva were diminished in size. The posterior side of the aortic leaflet of the mitral valve was the seat of a series of eccentrically shaped vegetations running in a line from the left anterior cusp of the aortic valve and extending below the free edge of the mitral valve. The aorta was diminished in caliber. The tricuspid and pulmonary valves were normal.

The liver was enlarged and of "nutmeg" appearance.

The spleen was markedly increased in size. A large light-pink infarct was present in the upper pole. The organ was firm and dark red.

The kidneys presented a few petechial hemorrhages. They were congested. The capsule stripped easily, leaving a smooth surface with many petechial hemorrhages.

Lantern slides were shown of sections through the aortic valve and the posterior leaflet of the mitral valve to illustrate the severe involvement of the former and the normal architecture of the latter.

#### DISCUSSION

C. DE LA CHAPELLE: We thought it important to present this case because of the infrequency of involvement of the aortic valve in rheumatic carditis without concomitant changes in the mitral valve. Emphasis has always been placed on the frequency of involvement of the mitral valve, and rightly so. In a series of ninety-seven necropsies of cases with rheumatic valvular disease analyzed by Coombs, the mitral valve was injured in every case. As a result of this, Coombs concluded that in *all* cases of rheumatic carditis the mitral valve is injured. In Thayer's series of sixty-five consecutive examples of fatal rheumatism with acute endocarditis there was a frequency of 92 per cent. This figure related only to acute changes. However, when he included the cases in which there were evidences of preceding attacks, the mitral valve was affected universally. Hence, we thought it worth while to present a case of this nature and to add one more instance to the group of cases of rheumatic carditis without involvement of the mitral valve.

#### AN INSTANCE OF METASTATIC CALCIFICATION. DOMINIC A. DeSANTO.

By metastatic calcification is meant a condition in which calcium is mobilized from the reservoirs in the bones and is deposited in other tissues of the body. The term was originally employed by Virchow. Wells reviewed the subject and found twenty-nine cases reported in the German literature, to which he added one of his own. The condition occurred in osteomyelitis, in primary and secondary tumors of bone and in leukemias. Well's own cases occurred in a young adult with myelogenous leukemia.

The calcium is most often deposited in the wall of the left auricle, in the pulmonary vessels, in the renal tubules and in the gastric mucosa near the fundus. At all of these sites, Wells pointed out, there is a change in the acidity of the tissue fluids in an alkaline direction, because at these sites acids are excreted (carbon dioxide and hydrochloric acid). The calcium is usually deposited in elastic tissues. These need not be previously injured, and this distinction between metastatic and pathologic calcification is important.

At Bellevue Hospital an example of metastatic calcification was encountered in a 19 year old girl whose history indicated that she had had chronic myelogenous leukemia for about two years. Post mortem, the wall of the left auricle was diffusely calcified. The pulmonary veins were calcified, and when the lung was sectioned, it imparted a grating sensation to the knife. The aorta and the visceral arteries were thrown into calcific ridges, giving the vessels a serrated appearance. Other findings were characteristic of myelogenous leukemia.

Microscopically, calcium was found deposited in the intima of the left auricle, which also showed leukemic infiltration. Calcium was present in the elastic tissue of the alveolar septums and in the intima of the pulmonary veins. Virtually every one of these vessels was affected. Calcium was also deposited in the renal tubules and throughout the aorta and visceral arteries, where it was present selectively in the inner elastic lamina.

## DISCUSSION

MAURICE N. RICHTER: Were there any changes in the bones in this case?

DOMINIC A. DESANTO: Unfortunately, we were not permitted to examine the long bones. We took pieces of ilium for examination. We could not find any evidence of decalcification in any one of the bones. The bone marrow, of course, showed the usual leukemic changes. When this condition has been described, it has usually been attributed to a decalcification of the bones as a result of metastatic carcinoma or primary sarcoma of the bone with mobilization of the calcium and secondary deposition at those sites that are, by their physical nature, predisposed to act as sites of deposition of calcium, namely the elastic tissues.

ALFRED PLAUT: What was the condition of the renal papillae—the tips of the pyramids?

DOMINIC A. DESANTO: I have not studied those particular sites. Grossly, I could find no evidence of calcification there. Microscopically, I did not focus my attention on that particular point.

PAUL KLEMPERER: Did the patient have radiotherapy?

DOMINIC A. DESANTO: The patient received radiotherapy two days before death. The period was so short that it would seem it could have had no effect on this pathologic condition, but it was immediately following the roentgen therapy that this peculiar sudden rise in the leukocyte count was observed. The patient had a count of 66,000, with a sudden rise to 1,200,000 two days after roentgen therapy. She had been treated with sodium cacodylate prior to that.

UNIVERSAL POLYPOSIS OF THE COLON, WITH CARCINOMATOUS TRANSFORMATION. L. LICHTENSTEIN.

Of the three cases presented, the first is represented by a specimen taken at autopsy from a man aged 60; it shows innumerable pedunculated, long-stalked polypi of the ascending and transverse colon, giving rise to five distinct primary carcinomas. The other two cases are represented by surgical specimens: one, from a man of 38 years, showing multiple papillomas of the descending colon with malignant degeneration in both the sigmoid and the rectum; the other, from a woman aged 51, shows diffuse polyposis of the ascending colon, with carcinomatous transformation of two polyps. Cases of polyposis adenomatosa diffusa, which appear to have a constitutional basis, often a familial incidence, are few in number, and are to be distinguished from the commonly encountered instances of isolated polyposis of the colon. Oberndorfer (cited by Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926) encountered a single case in a series of over ten thousand autopsies. Stämmeler-Schöttler (cited by Henke and Lubarsch) were able to collect from the literature (1923) approximately one hundred cases.

## DISCUSSION

PAUL KLEMPERER: Cases of multiple polyposis are rated in the literature as very rare. Oberndorfer lists only about one hundred cases; it is interesting that we should have had the opportunity to observe three cases in recent years.

NICHOLAS ALTER: I think they are more common than reported. I had a few cases of extensive and diffuse polyposis at the Post-Graduate Hospital, and it was interesting that some of these polypi had a tendency to become malignant. Definite invasions were seen in multiple places. The condition is often associated with ulcerative colitis, and in many cases of the latter disease I have observed polyposis with hyperplasia, which may be the causative agent and the starting point of the condition. Probably ulcerative colitis has its foundation in inflammatory hyperplasia, followed by metaplasia and possible transformation into cancer.

PAUL KLEMPERER: Diffuse polyposis of the type illustrated in the first specimen is not to be identified with numerous polyps in the colon. It only seems as though it were merely a matter of quantitative difference. If the colon is riddled with polypi as in the case shown, it is different from the others in which one can easily count the number of polypi. As these cases are frequently observed in younger people, they have a pathogenesis different from the polyps that occur as a result of inflammatory processes after dysentery or ulcerative colitis. The fact that there is a considerable number of familial cases further shows that this type is different in origin from the type following colitis. The occurrence of such universal polyposis is very unusual, and I think one should not identify it with the occurrence of several polypi in the intestine, which is, of course, a very common experience.

NICHOLAS ALTER: I have had specimens, one in particular, showing a very diffuse polyposis and cancer.

PAUL KLEMPERER: That shows that the experience of different persons varies.

#### ADAMANTINOMA WITH METASTASIS TO THE LUNGS. DAVID PERLA and JEFFERSON VORZIMER.

An instance of adamantinoma of the jaw with metastasis to the lung is reported. The bronchi of the lower lobe were markedly dilated, and their lumina were filled with a cast of the tumor tissue. In places, the parenchyma was invaded. It is suggested that the tumor tissue was aspirated into the lung from the primary tumor via the trachea and bronchial tree and grew primarily within the lumina of the bronchi. No similar metastatic lesion in the lung in a case of adamantinoma was found in the literature.

#### THE DIOXYPHENYLALANINE REACTION IN GENERAL PATHOLOGY. GEORGE F. LAIDLAW.

The dioxyphenylalanine reaction is specific for two kinds of cells, myelogenous leukocytes and melanoblasts. The reacting cell turns gray or black on a colorless ground. The reaction is easily performed, constant and inexpensive.

With myelogenous leukocytes, the dioxyphenylalanine reaction duplicates the Schultze-Winkler reaction, but it has some advantages. The dioxyphenylalanine reagent keeps better, and in the dioxyphenylalanine reaction the granules of the leukocyte stain a fast black. They can be counterstained in any way desired and the section mounted in balsam, whereas sometimes it is difficult to preserve and mount Schultze-Winkler sections without destroying the preparation.

For melanoblasts, the dioxyphenylalanine reaction is the only reliable specific reaction. It cannot be replaced by silver, as claimed by Heudorfer, nor by dimethylphenylendiamine, as stated by Kreibich and by Meirowsky. Our experiments confirm those of Bloch and his school that silver and dimethylphenylendiamine stain melanin and melanin only. If melanoblasts happen to contain melanin, they will stain with silver or with dimethylphenylendiamine; if they do not contain melanin, silver and dimethylphenylendiamine leave them unstained, but they may still stain with dioxyphenylalanine.

Lantern slides of the dioxyphenylalanine reaction were exhibited, showing melanoblasts in normal Caucasian skin and in Negro skin, their increase in number in a skin that has been tanned by the x-rays, and their absence in vitiligo. Slides of pigmented moles showed the correspondence of the dioxyphenylalanine reaction with the production of melanin, being greatest just beneath the epidermis and fading out in the deeper layers of the mole. Slides of a pigmented Recklinghausen-Lapennelephantiasis from the sacral region showed an increase in the number of melanoblasts in the pigmented epidermis, together with groups of Mongol cells deep in the corium. The Mongol cells were active melanoblasts,

reacting both to dioxyphenylalanine and to silver. The subject was a Caucasian girl, 16 years of age. Section from other areas, such as the gluteal fold, in the same case of elephantiasis, showed no Mongol cells.

In association with Lester Cahn, the speaker reported the constant presence of melanin and melanoblasts in human gums, a fact heretofore unknown.

The conclusion is that the dioxyphenylalanine reaction is a valuable aid to the pathologist in the identification of myelogenous leukocytes; it is indispensable in the study of the production of melanin, both in benign and in malignant melanoma. As a specific stain for melanoblasts, the dioxyphenylalanine reaction identifies the true melanoblast in the metastases of melanoma and distinguishes it from mere phagocytes. However, in the identification of melanoblasts, it should be remembered that the melanoblast is dioxyphenylalanine-positive only when actively producing melanin, and that it does not produce melanin continuously. A positive reaction is reliable; a negative reaction has no significance.

#### A SIMPLE TECHNIC FOR THE DIOXYPHENYLALANINE REACTION. S. N. BLACKBERG.

The published technic of the dioxyphenylalanine reaction requires considerable chemical and technical skill. This has prevented its general adoption. My associates and I have succeeded in simplifying the technic so that it can be carried out easily and successfully in any laboratory.

*The Stock Solution.*—Dissolve 0.3 Gm. of dioxyphenylalanine in 300 cc. of distilled water, cork tightly and keep in the refrigerator. When kept cold, the solution remains unchanged for many weeks.

*The Buffers.*—Dissolve 11 Gm. of disodium hydrogen phosphate in 1,000 cc. of distilled water.

Dissolve 9 Gm. of potassium dihydrogen phosphate in 1,000 cc. of distilled water.

Buffer the solution to  $p_H$  7.4 or 7.8 just before use. This can be secured with sufficient accuracy by mixing 50 cc. of the stock solution with 4 cc. of the potassium dihydrogen phosphate, and 12.5 cc. of the disodium hydrogen phosphate solution. The chief point is to have the  $p_H$  value above 7.3, below which the reaction does not take place. The  $p_H$  value may be 8.5 or even higher, giving a fast reaction.

*The Sections.*—Theoretically, frozen sections of fresh tissue should be used, but it is difficult to cut fresh tissue neatly. In practice, we follow Bloch's custom of hardening the tissue in 2 per cent formaldehyde for two or three hours. If fresh tissue is used, the sections are dropped from the knife directly into the buffered dioxyphenylalanine solution. Sections from formaldehyde-fixed tissue are rinsed in distilled water for five seconds or so and placed at once in the dioxyphenylalanine solution. Prolonged immersion in water is to be avoided, as it extracts the ferment rapidly.

*The Reaction.*—This takes place slowly at room temperature, and quickly at 56 C. It is usually conducted at 37 C. In about two hours the fluid turns reddish, then sepia brown. The first appearance of the sepia color marks the end of the reaction. A section is examined under the microscope. In a correct reaction, the melanoblasts and the leukocytes are gray or black, melanin retains its natural yellowish brown, and the collagen or other ground is colorless or pale gray. If it is desired to stain the leukocytes or melanoblasts more deeply, the section may be returned to the dioxyphenylalanine solution for another half hour or so.

Some tissues are sufficiently acid to lower the  $p_H$  value below 7.3, in which case the solution remains red, and the melanoblasts do not stain. When the tissue has been in formaldehyde, especially neutralized formaldehyde, the reaction is hastened, the fluid darkened and the ground often overstained. Since water cannot be used to wash out these disturbing elements, we change the dioxyphenylalanine solution for a fresh one after the first half hour, thus washing the sections with dioxyphenylalanine instead of water.

## DISCUSSION ON PAPERS OF DR. LAIDLAW AND DR. BLACKBERG

S. M. PECK: After Bloch had demonstrated his dioxyphenylalanine reaction, his work could be divided into two phases, one, that in which he showed that the dioxyphenylalanine reaction is a ferment reaction which takes place only in the pigment-building cells at the time when formation of pigment is active, and two, that in which he showed that dioxyphenylalanine is probably the propigment. It is agreed among workers on pigments that the term melanoblasts refers to those cells that are pigment-builders, whether they are ectodermal or mesodermal in origin, and that the term chromatophores should be applied only to those pigment-containing cells that phagocytose melanin. The mesodermal melanoblasts in man are but rudimentarily developed, as in the blue nevus and in the mongolian spots; they are also found in the eye, particularly in the choroid. Bloch's claim that there is a distinct relationship between the pigment-building activity of the cell and the intensity of the dioxyphenylalanine reaction was amply substantiated by some work that I did. I was able to show that after thorium X was applied to the skin, the dioxyphenylalanine reaction became increasingly positive up to a maximum intensity in about five days, and then gradually returned to a normal level. It remains fairly intense for four weeks after the application of thorium X. This was the first step in the formation of pigment. Actual melanin was formed later, and at the time when the greatest amount of pigment was seen in the cells, the dioxyphenylalanine reaction was on the wane. I was able to show, furthermore, that embryologic formation of pigment took place along similar lines. The first step was the presence of dioxyphenylalanine-positive cells at the place where formation of pigment was to take place.

Dr. Laidlaw was correct, I believe, in attributing a phasic formation of pigment to the melanoblasts, and that may be the reason why in the intensely pigmented Negro skin one sees relatively few dioxyphenylalanine-positive cells, but perhaps the phase of formation of pigment is much more rapid in the Negro's skin than in the Caucasian's skin.

The question of the origin of the dendritic cells has been much discussed. My research on this subject has led me to conclude that there are three possibilities: first, that all melanoblasts have a dendritic shape, but that the dendrites are seen only when they contain pigment or oxydase; second, that the melanoblasts can have either a dendritic shape or the ordinary basal cell form, and third, that the dendritic cells represent changes in shape of the ordinary basal cells which take place during intense formation of pigment. My studies on the formation of pigment after the application of thorium X have led me to believe that perhaps the last is the true state of affairs. Early after the application of thorium X, a section stained with hematoxylin and eosin, or even with silver nitrate, showed very few dendritic cells per field. But with the dioxyphenylalanine reaction there were in a low power field as many as 20 cells showing dendrites. Later on as melanin was formed, the number of dendritic cells seen with the silver nitrate reaction closely approached the number seen in the earlier sections.

Bloch and I have developed a technic for demonstrating oxydase in the leukocytes which is much simpler and much more practical than the Schultze-Winkler reaction. Furthermore, since melanin is formed in this reaction, the fine granules can be accentuated by means of the silver nitrate. It must be borne in mind that the reaction in the leukocytes should not be confused with the dioxyphenylalanine reaction. The former is not specific and can be obtained with epinephrine and pyrogallie acid.

I believe that we now have additional proof in support of Bloch's claim that the dioxyphenylalanine reaction is a ferment reaction and is absolutely specific. In collaboration with Sobotka and Kahn at Mount Sinai Hospital, I was able to show that the dioxyphenylalanine oxydase reacts only with the levorotatory dioxyphenylalanine. The dextro compound, however, is acted on as well as the levo by the leukocytes. This goes hand in hand with what is known of other ferment reactions, and since the levorotatory compound is the natural one, it is a further corroboration that the levo dioxyphenylalanine is probably the propigment.

I believe that the specificity of the oxydase for the levo compound will help us in demonstrating metastases of melanoma. While in the hands of an expert the dioxyphenylalanine reaction can be easily differentiated from that of the leukocyte, there is often a great deal of difficulty because of poor frozen sections. Therefore I would suggest that when there is any doubt, a reaction with both levo and dextro dioxyphenylalanine should be done. If the reaction is negative with the dextro and positive with the levo, one is dealing with melanoblasts.

ABNER WOLF: I should like to say a word about the experience I had with the dioxyphenylalanine reaction. Dr. Laidlaw was kind enough to demonstrate the reaction to me. It proved to be very simple. I applied it in three cases of anterior poliomyelitis, as the question arose whether some of the cells of the exudate were microglia cells or polymorphonuclear leukocytes. With the aid of the dioxyphenylalanine reaction performed with the simplified technic of Drs. Blackberg and Laidlaw, this point was easily determined.

PAUL KLEMPERER: Did the monocytes give any reaction?

GEORGE F. LAIDLAW: No.

#### SPHEROIDAL CELL CARCINOMA (SEMINOMA) OF THE EPIDIDYMISS NOT INVOLVING THE TESTICLE. A. A. EISENBERG and HARRY WALLERSTEIN.

A man, aged 48, noted progressive swelling with some pain in the right testicle, over a period of one year. There was no history of trauma or of venereal disease. The testicle and epididymis, when removed, showed a large, solid epididymis, measuring 3.5 by 3 cm. It was enclosed in a thick fibrous capsule, which separated it from the testicle. On section, the cut surface of the epididymis was brownish and solid, and resembled fleshy carcinoma-like tissue. There was an occasional area of softening. The testicle was small and compressed, and showed no gross areas of involvement. Microscopically, the epididymis was entirely converted into neoplastic tissue consisting of large, pale cells, some round, and others polyhedral in form. There was a distinct uniformity in the type of cell, which had a clear cytoplasm with a large nucleus containing considerable chromatin material, and in many cases filling the entire cell. The cells were arranged in large masses with no tendency toward alveolar formation. A small amount of stroma was present, and this was infiltrated with lymphocytes. A few necrotic areas were seen. The testis was separated from the tumor by a thick band of fibrous tissue, and was compressed, with many atrophied tubules. Several sections failed to show neoplastic involvement, though, properly to exclude this, the whole specimen would have to be sectioned, which was not done.

A search of the literature revealed twenty reports covering twenty-three cases of primary malignant neoplasms of the epididymis. Of these only two were of the type reported by us. One case was reported by Hinman and Gibson, and the other, by Coleman, Mackie and Simpson.

We believe the term "spheroidal cell carcinoma" to be preferred to any other, because it avoids the controversy regarding the origin of this tumor that is caused by the use of the term "seminoma" by Chevassu and "embryonal carcinoma" by Ewing.

This report with the results of a thorough search of the literature have been sent for publication to *Surgery, Gynecology and Obstetrics*.

#### INTERSTITIAL HYPERTROPHIC NEURITIS. ABNER WOLF.

A housewife, aged 40, (Neurological Institute, service of Dr. Frederick Tilney), complained of pain in both arms and legs, great difficulty in walking and weakness of both hands. The illness began in 1914 with a callus across the sole of the left foot at the level of the metatarsophalangeal joint. In 1921 numbness developed in the region of the callus and extended to the toes. Gradual weakness of the toes appeared, and within a year they were completely paralyzed, and eventually markedly contracted. This caused pain across the instep on walking. At about



the same time, a similar process began in the left hand and progressed until there was contracture unaccompanied by pain, however. In 1928 the right foot and hand became involved. The patient's previous history and that of the family were without obvious bearing on the condition.

The patient showed generalized muscular wasting, most marked in the distal parts of the extremities; weakness of the intrinsic musculature of the hands and feet, with bilateral "main en griffe," contractures and pes cavo-equinovarus, and involvement of the flexors and extensors of the wrists and fingers and of the quadriceps extensor femoris and anterior tibial groups of muscles. The electrical reactions in the muscles of the right arm and leg indicated degeneration; such muscles as the biceps and tibialis anticus did not react to the faradic current. A number of the peripheral nerves were thick and easily palpable, notably the posterior auricular, median and ulnar of both upper extremities, both radials, the right perineal and posterior tibial, and both superficial perineal nerves. Deep pressure on the nerve trunks caused no pain. The head and trunk were normal. There were diminished appreciation of pain, temperature and touch of a glove, and the stocking type of distribution in all four extremities. Vibratory sensibility, as well as sense of position and movement, were much diminished in both feet. No astereognosis was noted. The fundi and the visual fields, the pupils, and the rest of the cranial nerves were normal. The intelligence was normal, with good memory and insight, and there was no speech defect.

On Dec. 15, 1930, a small portion of the posterior auricular nerve was excised. It was markedly thickened, measuring 4 mm. in diameter, and very firm. It was studied by means of cross-sections stained in various ways.

The epineurium, moderately and somewhat irregularly thickened, was composed of broad bands of collagen that became somewhat more delicate at its inner surface and were arranged in horizontally concentric layers. Some of the bands toward the outer edge were rather glistening and homogeneous, as if partially hyalinized. A considerable number of elongated, fibroblast nuclei lay between the fiber bundles, their long axes parallel to the concentric collagen bands. Those toward the outer surface of the epineurium were compressed, condensed and elongated, while of those toward its inner surface, many were more oval and vesicular.

The perineurium appeared myxomatous. Scattered, compact bundles of collagen running in the longitudinal direction were present in moderate numbers. Between these, and between them and both the epineurium and the endoneurium, was a loose, weblike mass of delicate fibers interlacing, in all directions and staining often indefinitely. These stained faint blue with Mallory's connective tissue stain, reddish in van Gieson's stain and faint tan with phosphotungstic acid-hematoxylin. On their surfaces, especially where they lacked definition, and in some of the spaces between them, was a finely granular material, faintly pink in hematoxylin-eosin, reddish with Mallory's connective tissue stain and faint gray in Laidlaw's silver stain for connective tissue. In Mayer's mucicarmine stain, no mucin was demonstrated, although occasionally some of the fine fibers took a reddish tinge. Lying in the strands of the loose web were a few fibroblasts. Most of them were rather elongated and had elongated nuclei with a little chromatin in coarse granules; a few were star-shaped, with nuclei more nearly spherical. The larger vasae nervorum in the perineurium showed moderate fibrosis. In many of them, the adventitia and most of the media seemed to have undergone the same myxomatous change as the perineurium. Many of the arterioles showed partial or complete hyalinization. There were a few scattered mononuclear leukocytes and lymphocytes in the perineurium.

The nerve bundles in this perineurial tissue were striking. In cross-section, they consisted of groups of spherical masses, each many times the diameter of an ordinary axon and its sheaths. These masses had a concentric, lamellated structure, looser toward the outer margin and compact internally. They were either empty or surrounded a myelin sheath and axon, or a naked axon. Each of these tubular structures showed from one to four longitudinally disposed nuclei,

varying in shape. Their chromatin was scanty and coarsely granular; they lay between the lamellae, and their structure was the same whether they lay abutting on the myelin sheath, on the internal surface of the ring, or toward the outer margin of the ring. Some investigators have referred to these formations seen in cross-section as "onion-bulbs," because of their concentric, lamellated structure.

The laminae took an intense stain for collagen. Toward the outer margin of the laminated structure, the laminae were often composed of contiguous, loosely arranged, longitudinal bundles of collagenous connective tissue. With the stains for reticulum, horizontal bands of reticulin could be seen in the same zone. This outer area of the lamellated structure likely represents a thickened band of endoneurium.

The inner laminae had greater circular continuity and were more compact. In most cases, they were directly continuous with the outer laminae, appeared collagenous and had the horizontal bands of reticulin. A few of the bulbs showed a more homogeneous central portion, clearly separated from the outer, thickened endoneurial layer. These homogeneous areas also took the collagen stain intensely, and with the immersion lens looked finely stippled, as if they had many fine longitudinal fibrils in their substance. The inner portions of the concentric structures, it is likely, arose from hypertrophy and metamorphosis of the Schwann sheaths. The many nuclei seen in a single cross-section would represent an increase in the number of Schwann nuclei, since, ordinarily, only one such nucleus is present between two nodes of Ranvier.

The myelin sheaths were greatly reduced in number. No fragmentation of the myelin was encountered. In the preserved sheaths, the myelin was concentrated toward the margins of the sheath and about the incisors of Schmidt-Lantermann. With the Mallory connective tissue and Masson's trichrome stains, the neurokeratin network was well preserved in many of the undegenerated sheaths, and was stained orange and separated from the inner margins of the concentric, lamellated structures.

The total number of axons was increased, although many of the "onion bulbs" contained none. There was, however, a large number of naked axons of varying caliber, mostly delicate, lying eccentrically in the bulbs and at their margins, which represented regenerating nerve fibers as well as persisting sympathetic and demyelinated fibers. Of the persisting axons in a central position, some appeared hypertrophied, others, shrunken.

Thirty nine cases of interstitial hypertrophic neuritis have been described in the literature, with fourteen autopsies and five biopsies. An extended report is to appear soon in the *Bulletin of the Neurological Institute of New York*. We believe that this is a primary disease of the Schwann apparatus, characterized by overgrowth and sclerosis, and that the infiltrative cells in the perineurium are incidental to the degeneration of the myelin sheath and axons. Syphilis, intoxications, and other exogenous and endogenous factors have not appeared consistently enough in association with the disease to postulate them as the underlying cause. The frequent familial occurrence and onset in early youth suggest a congenital condition.

#### DISCUSSION

ALFRED PLAUT: Is anything known about the early stages of this disease?

ABNER WOLF: This condition does not come under observation until it is advanced. The changes in the Schwann cells are the initial ones; they do not produce any early symptoms because the myelin sheaths are intact for a long time, and the axons are the last to go, so that symptoms do not arise until late.

ALFRED PLAUT: Do you call this "neuritis" from the general clinical standpoint only—not from the anatomic standpoint?

ABNER WOLF: Yes.

LEON H. CORNWALL: I saw this case, and have seen three others. This is the first time that I have seen sections of this tissue stained by the Laidlaw

method. Dr. Wolf did not say whether or not he accepted the explanation in the literature, that this process is initiated by the Schwann cells and only later participated in by the epineurium, the endoneurium and the perineurium. It seems to me that there may be some doubt whether the so-called "onion bulbs" are comprised of Schwann cell components only. It is somewhat important to determine that. The same point arises in the conception of regeneration. Dr. Hassin of Chicago has advanced the opinion that the nuclei seen in nerves following injury, which have always been considered nuclei of Schwann cells, are derived from the mesodermal sheaths of the nerves and not from the ectodermal constituents. He believes that the Schwann cells act as scavengers in the peripheral nervous system in a manner similar to that exemplified by certain glial cells in the central nervous system, and that, when degeneration of myelin occurs, these scavenger cells ingest the degenerated lipoid products, transport them to the blood channels, and then end their existence. He believes that the cells forming the cordons between which the regenerating axis-cylinders insinuate themselves are of mesodermal origin. It may be that the pathologic change here is initiated by the mesodermal rather than by the ectodermal constituents. I would not imply that I hold that view, but I think it is possible, and I am rather interested to know Dr. Wolf's opinion of the significance of the fibrils that are stained by the Laidlaw method.

ABNER WOLF: I think Dr. Cornwall's point is well taken. However, the presence of reticulum between these bundles of Schwann cells does not rule out their being the primary factor in the formation of the lesions of the disease. Both Nagoette and P. Masson, among others, are of the opinion that the Schwann cells can produce both reticulum and collagen. Nagoette, in his work on regeneration in peripheral nerves, claimed that bands of Schwann cells growing out of the regenerating peripheral stump are able to lay down collagen in the absence of any fibroblasts. Fibroblasts, in his opinion, only secondarily invade the Schwann bands after the newly formed endoneurium has completely surrounded the reformed Schwann sheaths. Masson, in a report on schwannomas soon to appear, is of the opinion that the Schwann cells take part in the laying down of connective tissue fibers in these tumors. If these cells are capable of elaborating such fibers, the appearances seen in the Laidlaw stain in interstitial hypertrophic neuritis are not inconsistent with the Schwann cell hypothesis as to the formation of the typical lesions.

#### AORTICOVENTRICULAR FISTULA WITH ENGRAFTED ACUTE BACTERIAL ENDOCARDITIS. MENDEL JACOBI and ABRAHAM HEINRICH.

A white boy, 1½ years old (presented at the meeting on Dec. 10, 1931), was hospitalized because of pyrexia and painful knee and elbow joints. The heart was enlarged; apical and pulmonic systolic murmurs were present. A loud, harsh, to and fro murmur was present at the midsternum. The blood culture was positive for *Streptococcus haemolyticus*. The child died on the following day.

On necropsy, the heart showed a patch of fibrinous pericarditis over the left ventricle and over the conus. The aortic valve was bicuspid, and both coronary orifices opened into the larger anterior cusp. Between these two and on the same level, an opening 0.6 cm. in diameter led into a fistulous tract, which extended obliquely downward, forward and to the right, opening into the right ventricle beneath the commissure between the right and left posterior pulmonic valve cusps. The tract narrowed toward the ventricle and was filled with gray-yellow thrombus. A triangular cavity in the posterolateral right ventricular wall communicated with the tract. The kidneys presented minute cortical abscesses.

Microscopically, the tract was composed of a peripheral zone of granulation tissue and a lumen containing polymorphonuclear leukocytes, streptococci and debris. The medial zone of granulation tissue was widest at the ventricular end.

We explain the congenital defect as follows: The distal bulbar swellings that make up part of the aortopulmonic septum and are also the origin of the

aortic and pulmonic valve cusps are irregular as to form and occurrence, in man. We can conceive of a persistent communication in this distal bulbar septum itself, distal to the aortic valves, and the pulmonic valves arising immediately distal to such a defect. In support of this opinion, the pulmonic valves were 0.8 cm. above the aortic valves as measured from the apex. Usually the two valves are on the same level.

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, April 11, 1932*

R. H. JAFFÉ, *President, in the Chair*

### IMPACTION OF A NEURO-EPITHELIAL CYST IN THE THIRD VENTRICLE OF THE BRAIN. CARL O. RINDER and PAUL R. CANNON.

A woman, aged 47, died as a result of the impaction of a cystic tumor in the foramen of Monro. The tumor was 15 mm. in diameter, was filled with translucent, gelatinous material and was attached to the right choroid plexus. The contents was lipochrome, and the lining epithelium was ciliated. Such tumors, usually described as "colloid cysts," are supposed to arise from the paraphysis.

The clinical symptoms extended over ten years. These were intermittent headaches, nausea, visual disturbances and sudden relief by changes of posture, owing, presumably, to a ball-valve action of the cyst. Finally, the cyst became impacted, and death occurred rapidly from acute internal hydrocephalus.

#### DISCUSSION

J. P. SIMMONDS: I have examined the brain of a patient whose symptoms duplicated those described. The convolutions of the brain were flattened, and there was a cyst of the choroid plexus.

A. A. ARKIN: Ecchinococcus cysts and cysticercus cysts of the choroid plexus deserve consideration.

G. W. HALL: The symptoms of patients with these cysts, though very characteristic, may resemble those of other conditions, such as migraine.

### APPENDICITIS IN MEASLES. I. DAVIDSOHN and JACOB M. MORA.

The literature on the histologic changes in the tonsils and in the appendix in the prodromal stage of measles and during the course of the disease was reviewed. A second case was reported with characteristic giant cells in the lining of the appendix.

#### DISCUSSION

R. H. JAFFÉ: Similar cells occur in the secondary lesions of syphilis. These giant cells are not specific for any one condition.

### EXPERIMENTAL FAT EMBOLISM OF THE MYOCARDIUM IN DOGS. S. A. SZUREK and Z. G. CZAJA.

The results of emboli of fat in the coronary arteries of dogs were reported, with a brief survey of the literature on fat embolism. Oil pressed out from adipose tissue of dogs was slowly injected into one or more divisions of the anterior branch of the left coronary artery, and the hearts were examined after intervals of from six hours to thirty days. Instead of localized obstruction at some place near the mouth of the pulmonary artery in the aorta, as occurs with spontaneous occlusion in the human heart, now so familiar to clinicians, the

obstruction in the hearts of these dogs was due to a fluid embolus, spreading widely in the capillary bed distal to the site of injection. This factor resulted in some differences between the infarcts produced and those made experimentally in other ways and studied by other observers. The adventitious fat and the changes connected with its ultimate disposal in the entire region infarcted were observed; phagocytosis of fat and of the detritus of blood and necrotic muscle were conspicuous, and it was also noted that some of the oil introduced was still present in the partly healed lesions as long as thirty days after its introduction.

After this study was finished in the spring of 1931, and a report prepared for publication, an article by Vance appeared, (*The Significance of Fat Embolism, Arch. Surg.* **23**:426, 1931). The streaky and "flamelike" hemorrhages that he found in the myocardium of human hearts caused by emboli of fat following injuries accompanied by fractured bones are of interest because similar hemorrhages were observed in the dogs' hearts. The comment made by Vance on an absence of reports of human fibrous myocarditis due to fat embolism is also pertinent to this study of experimental lesions, for in the dogs' hearts scars were formed.

STENOSIS OF THE SUPERIOR VENA CAVA DUE TO MEDIASTINAL TUBERCULOSIS.  
GEORGE MILLES.

Obstruction of the superior vena cava may arise from aneurysms, mediastinal growths of many kinds, enlarged lymph nodes and thrombosis. Edema and cyanosis of the head, neck and thorax to the level of the fourth or the sixth ribs are the important clinical symptoms. The edema is most conspicuous in the head and neck, and least so in the arms. The veins of the collateral circulation are prominent. The subjective symptoms are dyspnea, orthopnea, dizziness, fulness of the head and somnolence.

A white man, 42 years of age, an ex-prize-fighter, was first seen on Feb. 5, 1931. He complained of dizziness, dyspnea, orthopnea, swelling of the face and neck and sleepiness. He had been well until the summer of 1930, when he noted dyspnea on exertion, dizziness, orthopnea and drowsiness. At about the same time, his face and neck began to swell, and his collar size increased. The symptoms were slowly progressive, accentuated on exertion or stooping, and during the following months the swelling of the face and neck fluctuated in degree, depending on his physical activity. About December, 1930, choking spells and extreme orthopnea disturbed him at night, and watering of the eyes became almost constant. During this time he had a slight, rather insignificant cough and lost about 6 pounds (2.7 Kg.). Physically, he was well nourished and exceptionally well developed, of the short, stocky type. His face and neck were definitely edematous and dusky; his lips were slightly cyanotic, and the conjunctivae showed injection and were edematous. The jugular veins were distended, and prominent varices encircled the costal margins. Slight widening of the upper mediastinal dulness was noted on percussion. The blood pressure was 128 systolic and 78 diastolic in both arms. Laboratory examination revealed 10.5 Gm. of hemoglobin per hundred cubic centimeters of blood, 4,560,000 red cells and 5,350 white cells of which 40 per cent were lymphocytes. The results of the Wassermann and Kahn tests were negative. The blood chemistry and the urine were normal. The x-ray plates and fluoroscopic examination revealed a slightly widened upper mediastinal shadow, which was interpreted as a tumor; no evidence of aneurysm was found. Deep x-ray therapy caused no change in the clinical findings during the following months. However, the hemoglobin increased to 16.5 Gm. per hundred cubic centimeters of blood and the red count to 5,480,000. The patient was observed until November, 1931; then he began to complain of some increase in dyspnea, although objectively little change had occurred. The cause of the obstruction was thought to be a benign tumor, and in view of the hopeless outlook a mediastinal exploration was undertaken by Dr. Lindon Seed. Markedly dilated superficial veins were encountered when the skin was incised. The right internal mammary vein was about 8 mm. in diameter, and when it was opened, a stream

of blood spurted 20 cm. from the upper end. The mediastinum was widely exposed, but no tumor was found. The superior vena cava was distended, blue and thick-walled. The wound was closed. On the following day, the edema of the head and neck increased, the arms were edematous, and cyanosis and dyspnea were marked. Respirations became rapidly labored, the edema and cyanosis more pronounced and the patient died on the second day after operation.

Postmortem examination demonstrated marked dilatation of the superficial veins and of the internal mammary, azygous and superior epigastric veins. A cartilagenous, poorly defined mediastinal mass adherent to the lung encircled the superior vena cava about 4 cm. above the right auricle. The lumen in this region was 2 mm. in diameter; the wall was thickened to 7 mm. by dense connective tissue, and at the center of the encircling mass was cheesy necrotic material like a necrotic lymph gland. Above the stenosis, the blood vessel was dilated moderately. The other viscera had no changes.

Microscopic sections through the stenosis demonstrated a central region of caseation necrosis. About this was dense hyalinized connective tissue with diffuse and focal infiltrations by lymphoid and epithelioid cells, fibroblasts, a few Langhans giant cells and macrophages. No tubercle bacilli were found in sections stained by the Ziehl-Nielsen method. However, macrophages containing a few variable-sized, acid-fast granules were noted. A diagnosis of chronic fibrocaseous tuberculosis of a mediastinal lymph gland was made. The stenosis of the superior vena cava was caused by the scar tissues around a caseous, tuberculous mediastinal lymph gland.

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## BUFFALO SOCIETY OF PATHOLOGISTS

*Meeting of April 26, 1932*

KORNEL TERPLAN, *President, in the Chair*

### PNEUMONIC CHANGES IN A FIVE MONTHS' FETUS. MARGARET WARWICK.

The mother of the infant had an entirely normal pregnancy, with no infection of any kind. Labor was spontaneous, and she delivered herself of a male fetus, weighing 13 ounces (404.3 Gm.), and, according to her last menstrual period, of a little less than five months' gestation. This fetus apparently had been dead for a short time before birth, but showed nothing of interest on gross examination. Microscopic sections showed the only pathologic lesions present to be in the lung. Only bronchiolar structures corresponding to the primitive alveoli were present. In all these, forming together a glandlike alveolar structure, were large collections of pus cells, in some of which could be made out a few brownish granules. The tissue between the primitive alveoli showed very mild infiltration by pus cells. Gram stains made on the pulmonary tissue failed to demonstrate any bacteria. This is a case illustrating pneumonic changes in a five months' fetal lung apparently caused by aspiration of amniotic fluid, in which there may have been some meconium as shown by brownish granules in the pus cells. The mother made an uneventful recovery with no evidence of any inflammatory process. Control examinations on sections taken from lungs of the same fetal age did not show the findings described.

### CONGENITAL ENDOCARDIAL AND MYOCARDIAL FIBROSIS WITH CALCIFICATION. W. F. JACOBS.

The case was that of a new-born girl who survived birth approximately four hours; she was cyanotic at first and continued thus until death. Gestation and delivery were normal. There seemed to be, however, an excess of amniotic fluid.

The postmortem observations were typically those of chronic passive congestion. The heart was much enlarged. This enlargement was symmetrical, involving the ventricles only. The shape of the heart was distinctly bulbous. The walls were resilient, so that the organ was not unlike a thin-walled rubber ball that after invagination springs back into shape. Both ventricles were dilated; the walls were about 5 mm. thick. The endocardial surfaces of both ventricles showed diffuse fibrosis, and on the cut edges fibrous strands could be seen penetrating the muscle. There was an open foramen ovale, normal in outline, and a patent ductus arteriosus of normal length and width. Histologically, the endocardium showed diffuse fibrosis. Areas of fibrosis and calcific deposits were found in the muscle. No cellular infiltration indicating an inflammatory process was seen.

The endocardial fibrosis can be assumed to have developed on a mechanical basis, as described by Marie Hertel in cases of so-called "functional elastic parietal endocardial fibrosis." This endocardial fibrosis may be secondary to the changes demonstrated in the muscle. The cause of the muscle damage could not be determined. B. Fisher reported a case in which there were endocardial and myocardial lesions similar to this one. In his case, however, infiltration by white blood cells was seen, and the mitral valve showed also fibrous lesions.

#### BACILLUS FRIEDLANDER SEPTICEMIA. E. T. MUELLER.

A white man, 63 years old, was in good health until Feb. 21, 1932, when he had a severe chill lasting thirty minutes. After that he felt well until the middle of March when he again had chills, which came on almost daily. Physical examination revealed only enlarged cardiac dulness. Moderate leukocytosis was present. Blood specimens taken on several occasions gave pure cultures of Friedländer's bacillus. At autopsy, no evidence of pneumonia or pleuritis was found. There was a large abscess below the left diaphragm, together with septic infarctions of the spleen. Other abscesses were found in the pancreas and the prostate. Purulent leptomeningitis was also present, and septic emboli were discovered in slides from the myocardium. Smears and cultures taken from the pancreas, the prostate and the meninges yielded only Friedländer's bacillus.

#### CYSTITIS EMPHYSEMATOSA IN A DIABETIC PATIENT. S. SANES.

Necropsy revealed cystitis emphysematosa in a 62 year old, white woman. Other important findings included marked generalized arteriosclerotic changes, especially of the basilar and vertebral arteries, with calcification and narrowing of the lumen; focal malacia of the pons with gliosis, and marked atrophy of the pancreas. During life the patient showed frequency of urination. The blood sugar was 790 mg. per hundred cubic centimeters; the carbon dioxide-combining power, 28 mm. of mercury. Grossly, the bladder was distended with 400 cc. of smoky yellow, gas-containing urine. Its entire lining was covered with white, foamy gas vesicles of varying sizes. Marked hemorrhage was also present. The histologic picture corresponded to chronic recurring cystitis with marked inflammatory edema, infiltration by round cells, many eosinophils, plasma cells and polymorphonuclear leukocytes, extensive recent hemorrhage, and pronounced desquamation of epithelium. Foreign body giant cells and endothelial giant cells were also noted. Throughout all layers of the wall were gas cysts, which included distended lymphatic vessels and emphysematous tissue spaces. Smears from the surface of the bladder and from the gas vesicles showed only gram-negative, plump bacilli with rounded ends. A culture of *Escherichia vesiculiformis* was obtained from the urine. Cultures from the bladder, taken after the organ had been fixed, were negative. Bacterial stains revealed short, rounded bacilli in the tissues. Factors of etiologic and pathogenic significance in this case, including sex, chronic invalidism, distention of the bladder with residual urine, cystitis, bacteria and hyperglycemia, were stressed. Twenty-nine instances of cystitis emphysematosa in human beings and animals have been reported. Two of these cases occurred in diabetic subjects.

## HODGKIN'S DISEASE OF THE BREAST. K. TERPLAN.

A Jewish woman, 58 years of age, entered the hospital complaining of a lump and pain in the right nipple. The lump had been present for a year, growing gradually larger and involving the areola. The past history was negative. Physical examination revealed a small, hard, solitary node on the right side of the neck. The right breast showed an enlarged, deformed nipple with a lumpy involvement of the pigmented area about the nipple. Nodes were palpable both in the axillae and in the groins. They were soft, movable and bean-sized. In the hospital a radical amputation of the right breast was performed, with removal of the axillary nodes. Important laboratory findings included a red blood count of 5,120,000, 80 per cent hemoglobin, and a white count of 9,450, with an increased number of eosinophils, basophils and monocytes. The Wassermann reaction was negative.

Grossly, the breast showed an elongated and deformed nipple, which was firm and dark brown. The areola was dark brown. The skin was not desquamated, but irregular. On section, the tissue underneath the nipple and areola, surrounding the ducts, was grayish white, firm and nodular. The remaining mammary tissue appeared normal. The lymph nodes were small, bean-sized, not hard, and on section glistening gray. The histologic picture showed a typical Hodgkin's granuloma around the ducts. In one axillary lymph node was noted a small nodule in beginning fibrosis with changes typically those of Hodgkin's disease.

The literature disclosed only one report of Hodgkin's disease of the breast, that of a case in a 16 year old girl with involvement of several peripheral lymph nodes, described by Kuekens.

The carcinoma-like gross picture of Hodgkin's disease of the breast, resembling somewhat superficial carcinoma of the ducts (so-called Paget's disease), was pointed out. The question of the pathogenesis of the changes in the breast was discussed, and the origin in this case by way of a retrograde lymphogenous spread in connection with the changes found in the axillary lymph nodes was considered.

## CHANGES IN THE BRAIN IN A CASE OF FATAL INSULIN SHOCK. K. TERPLAN.

A diabetic, white boy, 16 years old, had been given, at home and in the hospital, 92 units of insulin within twenty hours for apparent diabetic coma. Following this, he showed severe generalized convulsions, tonic and clonic, with nystagmus and twitching of the right side of the face and of the tongue. The blood dextrose was 46 mg. per hundred cubic centimeters; the white blood count was 12,000, with 81 per cent polymorphonuclear cells; the temperature was 101 to 107 F. After treatment to combat the hypoglycemia had been carried out, the blood sugar rose to 190 and 162 mg. per hundred cubic centimeters. The patient, however, never regained consciousness, dying in three days.

The most important postmortem observation was that of extreme edema of the brain (weight, 1,535 Gm.) with marked injection of the veins in the distended leptomeninges and in the brain substance itself. The same swelling was also noted in the spinal cord, which was closely pressed to the pachymeninges. The subdural space was practically obliterated, and the ventricles were markedly diminished in size, compressed by the swollen brain substance. Almost no spinal fluid was present. The pancreas weighed 34 Gm.

Sections stained by Nissl's method showed very severe changes in different parts of the cortex, chiefly in the occipital and frontal lobes, the island of Reil and the hippocampal gyrus. The changes consisted of extensive colliquation of the ganglion cells (many pictures of so-called Nissl's *Zellveränderung* being present), especially in the third layer. In some places, this layer had almost entirely disappeared. The glia cells presented severe regressive changes of the so-called amoeboid type; the capillaries showed extensive swelling of the endothelial cells. The changes were specially present in the depths of the gyri. The motor region showed almost no destruction compared with the other cortical areas. The ganglion cells in the brain stem, medulla, spinal cord and basal ganglia were also much less damaged. The etiologic factors involved in the severe destruction



of the ganglion cells in hypoglycemic conditions, particularly the marked edema of the brain, were discussed. The literature was reviewed and only one similar observation—two cases by Wohlwill—was found. In Wohlwill's cases, however, the histologic changes were more diffuse, and, strangely enough, no edema was present. My case demonstrated most clearly the reciprocal relationship between true swelling of the brain and the amount of cerebrospinal fluid. Several ante-mortem attempts to obtain spinal fluid by lumbar and cisternal puncture were fruitless. Only a small amount of bloody fluid was procured—no real spinal fluid.

#### DISCUSSION

BYRON D. BOWEN: This patient's symptoms were unquestionably due to insulin shock produced by the injection of a large quantity of insulin when the patient was already having hypoglycemic symptoms brought on by exercise. Adequate treatment was not given until eighteen hours after the onset of symptoms. The restoration of the hypernormal glycemia together with a sedative controlled the convulsions, but did not restore consciousness. These points suggest that the changes in the brain produced by hypoglycemia may reach a point that is irreversible by dextrose.

## Book Reviews

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**Man and Medicine: An Introduction to Medical Knowledge.** By Dr. Henry E. Sigerist, Professor at the University of Leipzig. Introduction by Dr. William H. Welch, Professor of the History of Medicine, the Johns Hopkins University. Translated by Margaret Galt Boise. Cloth. Price, \$4. Pp. 340. New York: W. W. Norton & Company, Inc., 1932.

The original German edition was published by Georg Thieme in Leipzig in 1931 under the title "Einführung in die Medizin." The following review was printed in the ARCHIVES (12:519 [Sept.] 1931):

"The author is Sudhoff's successor as professor of medical history in the University of Leipzig. He addresses himself especially to young persons about to begin the study of medicine. His object is to give them needed insight into the task ahead of them by means of a simple, yet comprehensive, conspectus of medicine as a whole on a historical or a developmental basis. There are seven chapters. The first deals with the structure and function—*anatomy, physiology and psychology*—of normal man. The necessity for the physician to acquire a broad knowledge of man in all his relationships is stressed. The next four chapters discuss the patient and disease. The growth of the concept of disease, its course and causes are discussed with admirable clearness. Then there is a chapter on diagnosis, healing and prevention. The last chapter is devoted to the doctor himself and his relation to society. The book is written in a remarkably clear, direct and effective style. Typographically, the failure to indent the paragraphs may be confusing to the reader. The book is highly interesting and will be of great help to medical students at the beginning of their course. It is recommended to all who are interested in the development of medicine and especially to medical educators. Sigerist shows clearly that as the developmental relations of various subjects in the study of medicine come to be understood, the sense of discontinuity in the medical curriculum, especially in the first two or three years, tends to vanish. The book merits translation into English."

Not much needs to be added to this statement. Dr. Welch, in his foreword, emphasizes that the clear and flowing style has been well preserved in the translation by Miss Boise. The book has been translated into Swedish, and translations into French and Spanish are to follow. The author writes in his preface: "The books is . . . a first attempt to picture the whole structure of medicine in the frame of general culture. When it appeared in Germany a year ago I was pleasantly surprised to find that it was welcomed by a large public outside of medical circles. Now a visit to Baltimore . . . and a lecture tour through the United States, have impressed me with the fact that there is in America a similar interest in the cultural aspects of medicine. I am therefore encouraged to believe that the present translation will appeal to the laity, as well as to medical students and physicians, in the English-speaking world." Simultaneously with the appearance of this translation comes the announcement that the author has accepted the professorship of the history of medicine in the Johns Hopkins University on the retirement of William H. Welch.

**Human Cancer: Etiological Factors; Precancerous Lesions; Growth; Spread; Symptoms; Diagnosis; Prognosis; Principles of Treatment.** By Arthur Purdy Stout, M.D., Associate Professor of Surgery, College of Physicians and Surgeons, Columbia University; Attending Surgical Pathologist, Presbyterian Hospital, New York. Cloth. Price, \$10, net. Pp. 1007, with 331 original engravings. Philadelphia: Lea & Febiger, 1932.

The opening sentence of the preface reads: "This monograph, as its subtitle indicates, is an attempt to discuss the development and growth of all the different kinds of cancer in the human body. It has long been recognized that each

particular anatomical region of the body produces cancers which differ in many respects from those in other parts of the body. Therefore, this book deals with cancer by regions." However, tumors of the central nervous system, malignant or benign, primary or secondary, are not considered except mainly for brief references to Hodgkin's disease, leukemia and lymphosarcoma of the brain and spinal cord. The title of the book does not indicate the omission, and no mention is made of it in the preface.

The word cancer is used to include every type of malignant tumor. In order to avoid confusion, only those names of different tumors are used "which are current, most widely known and therefore recognized by the greatest number." This is a good rule for a book like the one under consideration, but to apply the word epithelioma as the only designation to practically every form of carcinoma arising in flat surface epithelium is not in accord with the best usage, at least by pathologists, and furthermore is undesirable because it tends to create a suspicion that somehow epithelioma differs radically from carcinoma. In the index of the book, squamous cell carcinoma of the breast, the bronchus, the conjunctiva, the larynx, the lip, the tongue, the urethra, the skin, and so on is not listed under carcinoma but under epithelioma, while under skin, larynx and the like, carcinoma is not given, but squamous cell epithelioma. This arrangement is awkward. The simplest way would have been to have used carcinoma as the standard term for all carcinomatous cancers.

After a general introduction of four pages come forty-seven chapters on cancers in various parts of the body except, as stated, the central nervous system. In general, the chapters follow this order: introduction; etiologic factors and precancerous lesions; the beginning, growth and spread of the cancers under consideration; the symptoms and signs, and the essential principles of treatment. There are 331 original illustrations of gross and microscopic appearances, almost all photographic (not engravings), and for the most part commendable. The value of certain photomicrographs of appearances under higher powers, magnification not stated, is questionable. The presentation is clear and orderly. The book contains in well organized form a large amount of reliable information about the various types of cancer in different parts of the body exclusive of the central nervous system.

**The Life of Edward Jenner, M.D., F.R.S., Naturalist and Discoverer of Vaccination.** By F. Dawtrey Drewitt, M.A., M.D., F.R.C.P. Cloth. Price, \$2. Pp. 127, with portrait. New York: Longmans, Green & Company, 1931.

And he stood between the dead and the living; and the pestilence was stayed.—Numbers XVI, 48.

Much has been written about Edward Jenner, mostly in the form of brief sketches in medical journals. The value of his work to humanity is recognized: he is one of its greatest benefactors. Of the formal biographies of Jenner, there has been only one, John Baron's, published in two volumes (1827-1838), the last fifteen years after Jenner's death, in which the "true and genuine lineaments of his mind should stand forth in all their fair and just proportions." Fortunately the records in Baron's work are an invaluable source of information about Jenner—true "memoirs pour service"—but obviously they themselves will be read by only a few. Drewitt's little book, which is based mainly on Baron's, gives an excellent short account of Jenner's life which should interest wide circles of readers. In short space the author succeeds in giving an accurate, comprehensive and winning picture of Jenner: his life as a country doctor, his observations in natural history, his establishment by simple experiments of vaccination against smallpox and his wisdom, simplicity and benevolence.

**Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung.** Von Prof. Dr. E. M. P. Widmark, Mediz.-chem. Institut der Universität Lund, Schweden. Neue Folge, Helt 11. Fortschritte der naturwissenschaftlichen Forschung. Herausgegeben von Prof. Dr. Emil Abderhalden. Paper. Price, 14 marks. Pp. 140, with 59 illustrations. Berlin: Urban & Schwarzenberg, 1932.

A practical and reliable micromethod for determining the presence of ethyl alcohol in the blood is described, based on the bichromate method. The micro-method has been used extensively in Sweden to determine alcohol in the blood for medicolegal purposes. The results of examinations of about seven hundred samples of blood in the course of routine investigation of traffic accidents are presented and discussed. Especially interesting cases are reported in detail. The determination of alcohol in the blood and urine in necropsies is also considered. The book is a highly important contribution to the study, scientific and practical, of the medicolegal problems of alcohol.

**General Bacteriology.** By Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago, and the Rush Medical College, Chicago. Tenth edition, entirely reset. Cloth. Price, \$6 net. Pp. 819, with 200 illustrations. Philadelphia: W. B. Saunders Company, 1931.

Not much need be said by way of introducing the tenth edition of this standard and popular textbook. The revision has brought the book well abreast with current knowledge. Variation, undulant fever, filtrable virus diseases and bacteriophage are some of the topics that have received special consideration. The question of "nomenclature has been dealt with perhaps rather summarily," says the preface. "Generic names that have come into quite general use, such as *Salmonella*, *Mycobacterium* and *Brucella*, are tentatively employed, and even *Eberthella* has been somewhat reluctantly introduced. Convenience rather than strict rules of taxonomy seems likely to govern the naming of bacteria until a general nomenclatural system can be worked out by international agreement." Although the number of pages now is 819, this increase does not indicate any relaxation in the concise clearness, the judicial restraint and the remarkable accuracy of the presentation. In these respects the book continues to be a model.

## Books Received

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COLLECTED REPRINTS FROM THE LABORATORIES OF THE MOUNT SINAI HOSPITAL, NEW YORK. By Louis Gross, M.D., Director, 1931.

MEDICAL ASPECTS OF OLD AGE. BEING A REVISED AND ENLARGED EDITION OF THE LINACRE LECTURE, 1922. By Sir Humphrey Rolleston, Bart., G.V.C.O., K.C.B., M.D., Hon. D.Sc., D.C.L., LL.D., Regius Professor of Physic in the University of Cambridge, Physician-in-Ordinary to the King, Sometime President of the Royal College of Physicians of London. Price, \$3. Pp. 205. New York: The Macmillan Company, 1932.

MAN AND MEDICINE: AN INTRODUCTION TO MEDICAL KNOWLEDGE. By Dr. Henry E. Sigerist, Professor at the University of Leipzig. Introduction by Dr. William H. Welch, Professor of the History of Medicine, the Johns Hopkins University. Translated by Margaret Galt Boise. Price, cloth, \$4. Pp. 340. New York: W. W. Norton & Company, Inc., 1932.

HUMAN CANCER: ETIOLOGICAL FACTORS; PRECANCEROUS LESIONS; GROWTH; SPREAD; SYMPTOMS; DIAGNOSIS; PROGNOSIS; PRINCIPLES OF TREATMENT. By Arthur Purdy Stout, M.D., Associate Professor of Surgery, College of Physicians and Surgeons, Columbia University; Attending Surgical Pathologist, Presbyterian Hospital, New York City. Price, cloth, \$10, net. Pp. 1,007, with 331 original engravings. Philadelphia: Lea & Febiger, 1932.

STUDIES IN NUTRITION: AN INQUIRY INTO THE DIET OF FAMILIES IN CARDIFF AND READING. By E. P. Cathcart and A. M. T. Murray, assisted by M. Shanks. Medical Research Council, Special Report Series, no. 165. Price, 6 pence, net. Pp. 28. London: His Majesty's Stationery Office, 1932.

LA MALADIE DE BOECK. SARCOIDES CUTANÉES BÉNIGNES MULTIPLES. Par le Docteur A. Kissmeyer, Médecin de L'Institut Finsen. Préface du Dr. J. Darier. Pp. 147, avec 67 illustrations et planches. Paris: Masson et Cie, 1932.

REPORT OF THE NEW YORK STATE VETERINARY COLLEGE AT CORNELL UNIVERSITY FOR THE YEAR 1930-1931. Legislative Document (1932) no. 19. Pp. 226. New York: Burland Printing Company, Inc., 1932.

VACCINATION PRÉVENTIVE DE LA TUBERCULOSE DE L'HOMME ET DES ANIMAUX PAR LE BCG. Rapports et documents provenant des divers pays (La France exceptée). Transmis à L'Institut Pasteur en 1932. Par A. Calmette et al. Pp. 366. Paris: Masson et Cie, 1932.

## CALCIFICATION OF THE MYOCARDIUM IN A PREMATURE INFANT

MORTIMER DIAMOND, M.D.

CHICAGO

Though pathologic calcification is not uncommon, calcific changes in the myocardium are rare. Forty-four cases have been reported, but the records of only forty-one, including the case presented here, could be obtained, and of these many were incomplete. Of these forty-one cases, twenty occurred in males and twelve in females; in the reports of the nine remaining cases, the sex was not given. In twenty-one instances, the ages ranged from 21 to 60 years. The youngest patient was the one whose case is reported in this paper, namely, a 26 weeks premature infant; the oldest was aged 86 years.

The accompanying table shows that calcification of the muscle fibers is secondary to necrosis or to certain forms of degeneration, except fatty degeneration. In only one case (Sturzenegger's<sup>1</sup>) was necrosis not described, but it is possible that with decalcification necrotic muscle fibers would have been seen. The relationship between fatty degeneration and calcification is shown by Robin,<sup>2</sup> Hart,<sup>3</sup> Pappenheimer<sup>4</sup> and Roth<sup>5</sup> to be of no significance. This is in accordance with my findings. Only Stumpf<sup>6</sup> observed some muscle fibers with both fatty degeneration and calcification, and even he concluded that the relationship is unusual.

The causes for the necrotic (degenerative) changes in the muscle fibers were used as a basis for classifying the cases summarized in the table. This table brings out three main groups, namely, the vascular, the inflammatory and the toxic. These will be discussed in detail later, although I may say at this point that there is not a pure case of calcium metastasis involving the cardiac muscle fibers reported in the literature.

The case now reported is interesting because, as mentioned, it occurred in the youngest of all the subjects so far observed, and because it illustrates clearly the pathogenesis of the calcification.

From the Department of Pathology, Cook County Hospital; Dr. R. H. Jaffé, Director.

1. Sturzenegger, E.: Beitr. z. path. anat. u. z. allg. Path. **78**:85, 1927.
2. Robin, A.: Bull. et mém. Soc. méd. d. hôp. de Paris **2**:99, 1885.
3. Hart, C.: Frankfurt. Ztschr. f. Path. **3**:706, 1909.
4. Pappenheimer, A.: Proc. New York Path. Soc. **10**:129, 1910.
5. Roth, M.: Cor.-Bl. f. Schweiz. Aerzte **9**:226, 1884.
6. Stumpf: Centralbl. f. allg. Path. u. path. Anat. **25**:801, 1914.

## REPORT OF A CASE

The mother, a 24 year old primipara, white, married, was admitted to the Cook County Hospital on Oct. 7, 1931. She was six and a half months pregnant, in the second stage of labor, and was delivered of a female fetus that died thirty minutes after birth with signs of cardiac failure.

At autopsy, the heart weighed 16 Gm. The epicardium was thin, transparent and smooth. Three fourths of the apex was formed by the left ventricle, the wall of which measured 6 mm., while that of the right ventricle measured 3 mm. The myocardium was light purple-gray with some yellow, and was soft. The left ventricle was slightly dilated, its transverse diameter being equal to its vertical diameter, which was 28 mm. The endocardium of the left ventricle was thin and transparent, but the trabeculae carneae were slightly flattened. None of the valves showed changes. In striking contrast to the thin, smooth and elastic aorta, the first measurement of which was 19 mm., the wall of the right auricle was rigid, owing to the presence of yellowish-white deposits, which to a large extent had



Fig. 1.—Roentgen picture of the fresh heart showing the linear deposits of calcium in the right auricle and irregularly scattered deposits of calcium in the walls of both ventricles.

singled out the trabeculae carneae, making them appear as distinct, yellowish-white, linear bundles. The auricle measured 21 by 15 by 12 mm.; its wall was as much as 3 mm. in thickness, and its endocardium was smooth. The appendage of the right auricle was soft. The foramen ovale was anatomically patent.

The other anatomic findings were: marked intermeningeal hemorrhages, especially in the region of the temporal lobes; marked edema of the brain; edema and hyperemia of the leptomeninges; subaponeurotic and subcutaneous hemorrhages of the scalp; marked edema of the scalp and face; passive congestion of the liver, spleen and kidneys; edema of the lungs; ascites, and prematurity.

In order to determine the extent of the calcification more exactly, an x-ray picture of the fresh heart was taken (fig. 1). Here one plainly sees the marked involvement of the right auricle and irregularly scattered deposits of calcium in the walls of both ventricles.

*Microscopic Observations.*—In the right auricle there were extensive deposits of calcium, which appeared in the form of coarse and branched trabeculae (fig. 2).

In many places, the calcification involved the entire thickness of the wall from the epicardium to the endocardium, which was markedly thickened. The remaining muscle fibers were narrow, particularly those in close proximity to the deposits of calcium, and appeared as slender bands with elongated nuclei. In some places near the fibrous ring of the tricuspid valve, there were interstitial accumulations of small round cells, which were arranged about thin-walled capillaries. The interstitial accumulations were surrounded by fibrillar connective tissue, which extended between the adjacent muscle fibers. Some of the muscle fibers thus became isolated and separated from the other fibers.

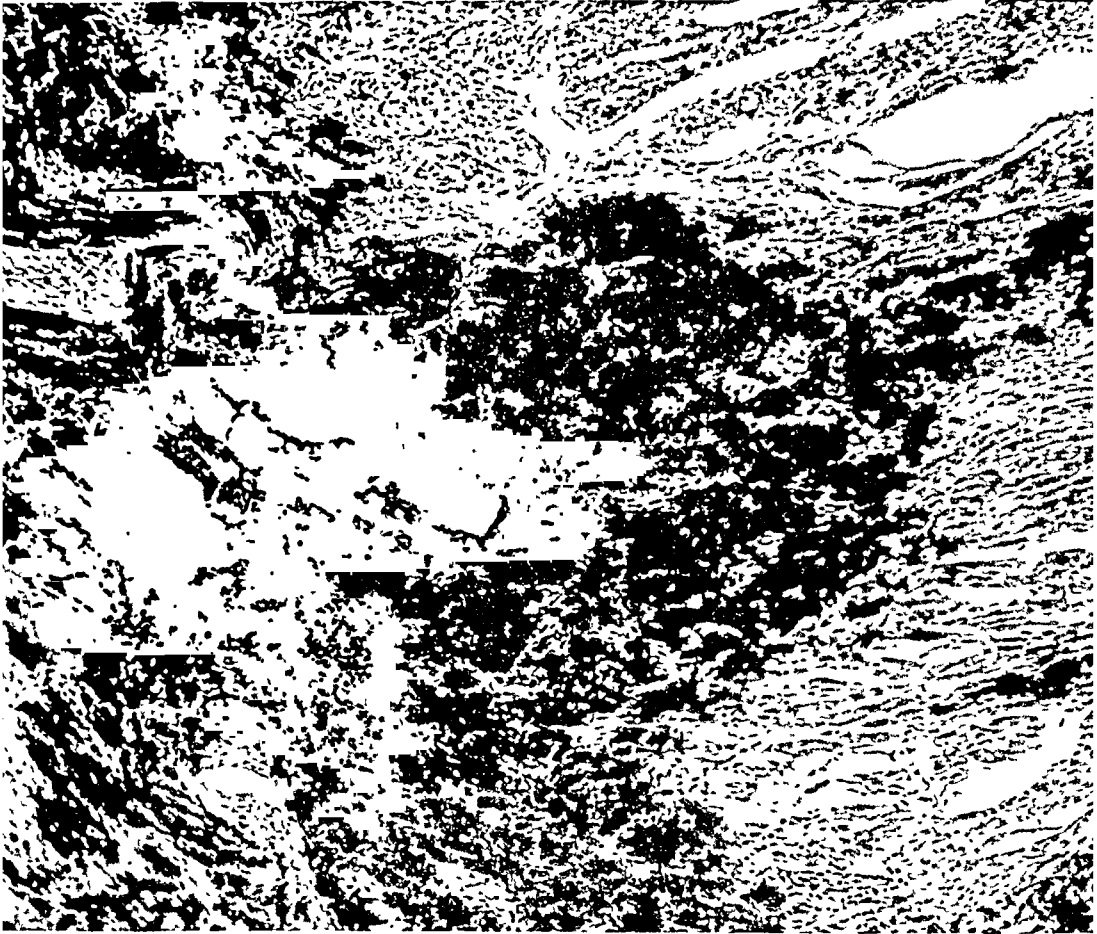


Fig. 2.—Wall of right auricle showing extensive calcification. The origin of the deposits of calcium from the muscle fibers is well shown in the upper left corner of the picture. Formaldehyde fixation; celloidin section; Kossa stain counterstained with alum carmine; reduced from a magnification of  $\times 120$ .

In the right auricular appendage practically all the muscle fibers had been substituted by calcium, and the derivation of the calcium trabeculae was clearly shown. Not only did the calcium trabeculae imitate the outlines of the muscle fibers, but they also showed their characteristic arrangement. During decalcification of the section with hydrochloric acid, gas bubbles were formed. The decalcified muscle fibers were seen to be without striations, their nuclei were irregular, and the fibers appeared swollen. Fat stain did not show any sudanophil material. The calcified



structures did not show any relation to the elastic fibers, and no iron was found in the sections. Between the calcified muscle fibers there was a moderate amount of loose, fibrillar connective tissue, which in places became densely infiltrated by small round cells.

Scattered throughout the myocardium of the right ventricle were small, irregular areas of increase of the interstitial tissue. Some of these areas were composed of fibrillar connective tissue, while others were rich in small, round cells. The muscle fibers were fairly well preserved.

The changes in the left auricle were similar to those described in the right auricle, although they were less pronounced. There was an occasional isolated muscle fiber which was calcified and surrounded by fibrillar connective tissue, loosely infiltrated by small round cells. There were, however, also places in which larger groups of muscle fibers were completely calcified.

There were many small interstitial accumulations of small round cells in the anterior wall of the left ventricle and an occasional focus of calcification of the muscle fibers, which was much less pronounced than in the auricles. These infiltrations occupied chiefly the central parts of the myocardium.

In the septum, the interstitial infiltrations were scanty, being found especially beneath the endocardium. When present, they were associated with calcification of the muscle fibers.

In the center of the papillary muscle of the left ventricle there was a large deposit of calcium in the form of coarse trabeculae. The deposition was surrounded by connective tissue and loosely scattered small round cells. Some of the muscle fibers near the calcified areas showed an increased affinity to the eosin stain.

The aorta and pulmonary artery did not show any abnormal changes.

The suprarenal cortex was well developed, and in particular the glomerular zone was prominent. In the reticularis, near the medulla, there were small round or cuboidal calcium deposits that reached the size of a cortical cell. In some places, the cortical cells accumulated and formed small pleomorphic nodules (cortical adenomas).

#### COMMENT

It is evident that the calcification of the myocardium in this case was secondary to degenerative changes in the muscle fibers. Areas of calcification of the interstitial tissue as described by Robin,<sup>2</sup> Roth,<sup>5</sup> Askanazy,<sup>7</sup> Scholz,<sup>8</sup> Hedinger (case 2<sup>9</sup>), Geipel<sup>10</sup> and Siebenmann<sup>11</sup> were not found. There were some groups of muscle fibers showing hyaline degeneration that were surrounded by normal myocardium or were adjacent to calcified muscle fibers. In other places fibrous tissue was seen surrounding them. Further, areas of calcification might be seen surrounded either by normal muscle fibers or by scar tissue. There were also areas of muscle fibers separated from each other by scar tissue, but this finding is explained by the fact that the section was cut tangentially through the interstitial proliferated stroma surrounding areas of calcification. In some places there were accumu-

7. Askanazy: *Festschr. z. Feier d. 60 Geburtst. v. M. Jaffe*, 1901, p. 187.

8. Scholz, T.: *Arch. Int. Med.* **34**:32, 1924.

9. Hedinger: *Verhandl. d. deutsch. path. Gesellsch.* **11**:295, 1907-1908.

10. Geipel, P.: *Fortschr. a. d. Geb. d. Röntgenstrahlen Hamb.* **34**:311, 1926.

11. Siebenmann, F.: *Inaugural Dissertation*, Basel, 1909.

lations of round cells, but polymorphonuclear leukocytes were not found. The former were found in relation to the calcified masses. The sections stained with sudan III showed clearly that fatty degeneration played no rôle in the calcification of the muscle fibers. In brief, one could distinguish three distinct processes: hyaline degeneration of the muscle fibers, deposition of calcium and reactive proliferation of the stroma. These changes could be correlated as follows: The degeneration of the muscle fibers was apparently primary and was followed by calcification. The calcium acted as a foreign body and caused a reactive proliferation of the stroma. This conception is also held by Wiechert,<sup>12</sup> Pappenheimer,<sup>4</sup> Hart,<sup>3</sup> Fischer<sup>13</sup> and others.

The question now arises as to the cause of the degeneration. The degeneration could not have been due to vascular changes, since macroscopically and microscopically the aorta and coronary arteries were thin-walled, and the coronary arteries everywhere were patent. An infectious origin also could be ruled out, because there were no signs of inflammation in the heart save those secondary to the calcification. It was not a case of calcium metastasis (Virchow<sup>14</sup>); for the skeleton showed no abnormalities on roentgen examination, the muscle fibers that were calcified were degenerated, and finally the calcium deposits had no relationship to the coronary arteries. Thus it is most likely that the cause of the degeneration was toxic. There were no evidences of infection in the child; therefore one has to assume that the toxic agent might have come from the mother. I attempted to obtain information concerning the presence of some infection or intoxication during the pregnancy, but unfortunately I was unable to do so because the mother had left the hospital and could not be located.

There are three cases reported in the literature that resemble the one now reported in the matter of age: Jacobsthal's,<sup>15</sup> which differs because definite inflammation was present in the anterior papillary muscle; Fischer's,<sup>13</sup> different in that both endocarditis and myocarditis were present, and Sturzenegger's,<sup>1</sup> different because the infant had fetal meconium peritonitis as the cause for the degeneration of the myocardium.

Other cases of toxic necrosis of the myocardium have been reported by Coats,<sup>16</sup> Wiechert,<sup>12</sup> Pappenheimer,<sup>4</sup> Tilp<sup>17</sup> and Liebscher,<sup>18</sup> but in these the patients were from 26 to 48 years of age. In this connection it may be mentioned that Iff<sup>19</sup> recently reported a case of a new-born

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12. Wiechert, A.: Inaugural Dissertation, Marburg, 1907.

13. Fischer, B.: Frankfurt. Ztschr. f. Path. **7**:83, 1911.

14. Virchow: Virchows Arch. f. path. Anat. **8**:108, 1855; **9**:618, 1855.

15. Jacobsthal: Virchows Arch. f. path. Anat. **159**:361, 1900.

16. Coats: Glasgow M. J. **4**:433, 1872.

17. Tilp: Verhandl. d. deutsch. path. Gesellsch. **15**:471, 1912.

18. Liebscher, C.: Prag. med. Wchnschr. **27**:181, 1902.

19. Iff, W.: Virchows Arch. f. path. Anat. **281**:377, 1931.

child in whom the media and to a lesser extent the intima and adventitia of the large and medium-sized arteries were calcified. He similarly explained the changes on the basis of toxic necrosis of the media with secondary calcification.

#### REVIEW AND CLASSIFICATION OF CASES IN THE LITERATURE

From the table it can be seen that the causes of degeneration of the muscle fibers fall into three groups. The first of these is the vascular,

#### *Etiology of Primary Degeneration in Calcification of the Myocardium*

<b>I. Vascular Group (coronary Occlusion)</b> <b>A. Coronary sclerosis</b> 1. Burns 2. Robin 3. Askanazy 4. Scholz <b>B. Coronary thrombosis</b> 1. Stumpf 2. Davidson <b>C. With granular kidney</b> 1. Heschel 2. Hedinger (case 2) 3. Geipel 4. King	<b>II. Inflammatory Group</b> <b>A. Pyogenic abscess</b> 1. Hardrichs <b>B. Tubercle</b> 1. Püschman <b>C. Myocarditis</b> 1. Jacobsthal (etiology?) 2. Hart (chronic syphilis) <b>D. Myocarditis and endocarditis (rheumatic)</b> 1. Fischer 2. Krayn <b>E. Pancarditis (rheumatic)</b> 1. Cutler and Sosman (case 1) <b>F. With pericarditis</b> 1. Bordenave (cause?) 2. Simmons and Watson (pneumococic) 3. Burnet (pneumococic) 4. Drummond (pneumococic) 5. Cutler and Sosman (case 2 pneumococic)	<b>III. Toxic Group</b> <b>A. Infectious toxemia (chronic sepsis)</b> 1. Chronic bronchitis (Coats, case 1) 2. Relapsing fever with pyemia (Coats, case 2) 3. Chronic tuberculosis (Liebscher) 4. Infection with <i>B. paratyphosus</i> (Wiechert) 5. Gangrenous appendicitis with suppurative peritonitis, hepatic abscess, etc. (Pappenhefmer) 6. Chronic bronchiectasis empyema (Tilp) <b>B. With destruction of bone</b> 1. Chronic osteomyelitis of hand (Roth) 2. Chronic osteomyelitis of long bones (Siebenmann) <b>C. Noninfectious toxemia</b> 1. Chronic lead poisoning (Langerhans) 2. Eclampsia (Roessle) 3. Meconium peritonitis (Sturzenegger) 4. Bichloride of mercury poisoning (Reuther)
<b>IV. Unclassified Group</b> 1. Rokitsansky 2. Topham 3. Hedinger (case 1) (toxic—cause?) 4. Diamond (toxic—cause?)	<b>V. Group, the Records of Which Were Unobtainable</b> 1. Allen 2. Benjaminovich (2 cases) 3. Waldorp	

and in it are included all those cases in which the degeneration followed coronary occlusion whether due to sclerosis, thrombosis or spasm. In four cases, coronary sclerosis was the important factor.

Burns<sup>20</sup> reported the case of a man with advanced arteriosclerosis, thrombosis of the left auricle, marked coronary sclerosis and irregular calcified areas in the left ventricle. Robin<sup>2</sup> reported the case of an 85 year old man who died from right hemiplegia, and who at autopsy showed sclerotic aorta and coronary arteries, a calcareous plaque in the

20. Burns, A.: Some of the Most Frequent and Important Diseases of the Heart, Edinburgh, Bryce & Company, 1809, p. 340.

endocardium of the left side of the interventricular septum and, microscopically, in the left ventricle, in areas of fibrous tissue, around partially obstructed arterioles, "fine strands which gave gas when treated with hydrochloric acid." Askanazy<sup>7</sup> wrote on a case of a woman 36 years old with coronary sclerosis and irregular areas of calcification in the wall of the left ventricle. Finally, Scholz<sup>8</sup> reported a case of massive calcification of the left ventricle especially at the apex in a man 74 years old, whose left coronary artery was almost occluded by calcific plaques.

The cases of Stumpf<sup>6</sup> and Davidson<sup>21</sup> illustrate coronary thrombosis as the cause of the necrosis.

Four cases are associated with granular contracted kidneys, and since myocardial degenerations in these cases are often due either to arteriosclerosis or to coronary spasms, they have been included in the vascular group. The first was that observed by Heschl<sup>22</sup> in 1861; the patient had classic Bright's disease and showed at autopsy contracted kidneys. Calcium carbonate was found in broken up heart muscle fibers. He was incorrect in believing this to be a case of calcium metastasis in spite of the absence of destruction of bone. The second, Hedinger's<sup>9</sup> second patient, had contracted kidneys and calcified masses in the left and right ventricles. The third case (Geipel<sup>10</sup>) concerned a 15 year old girl who died in uremia and showed calcification of the right auricle, septum and anterior papillary muscle. The coronary arteries were slightly involved also. The last, described by King,<sup>23</sup> was the case of one who died in uremia and in whom microscopically irregular areas of calcification were found.

In the second group of cases true inflammatory conditions were present, including: a myocardial abscess with calcified wall (Hardrichs<sup>24</sup>), a calcified tubercle in the myocardium (Püschman<sup>25</sup>) and focal myocarditis with calcium in the degenerated muscle fibers (Jacobsthal<sup>15</sup>). In Hart's<sup>3</sup> case there was congenital syphilis and recent verrucous endocarditis, and microscopically there were numerous areas of focal inflammation with infiltration by leukocytes as well as by monocytes. Calcium was found in destroyed muscle fibers. Hart believed that amyloid, hyaline and glycogenic degenerations may be the basis for calcium depositions. The finding of hyaline degeneration in my case supports this theory, and, indeed, Beneke and Bönning<sup>26</sup> showed a relationship between amyloid and calcification. Fischer's<sup>13</sup> case was one of fetal

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21. Davidson, T. W.: *Brit. M. J.* **1**:212, 1928.

22. Heschl: *Oesterr. Ztschr. f. prakt. Heilk.* **7**:49, 1861.

23. King, V. de P.: *Ann. Int. Med.* **2**:936, 1929.

24. Hardrichs: *M. Rec.* **18**:552, 1880.

25. Püschman: *Inaugural Dissertation*, Leipzig, 1896; abstr., *Centralbl. f. allg. Path. u. path. Anat.*, 1897.

26. Eeneke and Bönning: *Beitr. z. path. Anat. u. z. allg. Path.* **44**:362, 1908.

endocarditis and myocarditis, and microscopically many varieties of cells including leukocytes were found in the myocardium as was calcium in the degenerated muscle fibers. In Krayn's<sup>27</sup> case there was a double mitral lesion due to old rheumatic endocarditis probably associated with myocarditis, and calcium was found deposited in broken up muscle fibers. Cutler and Sosman<sup>28</sup> reported a case (1) of a 26 year old woman with recurrent attacks of rheumatic fever and who at autopsy gave evidence of pancarditis with calcification of the papillary muscles, pulmonary and mitral leaflets and pericardium.

In each of the next six cases listed in the table (Bordenave,<sup>29</sup> Simmons and Watson,<sup>30</sup> Burnet,<sup>31</sup> Drummond,<sup>32</sup> Lucas<sup>33</sup> and Cutler and Sosman<sup>28</sup> [case 2]) there was found calcification of the pericardium with the calcification extending into the ventricles, being due to myocarditis associated with the pericarditis or to toxic degeneration of the myocardium due to the pericarditis.

The third group consists of cases of toxic necrosis, infectious or noninfectious, of the muscle fibers. The infectious subgroup includes cases of chronic bronchitis (Coats<sup>16</sup> [case 1]); relapsing fever with pyemia and abscesses in the lungs, parotid glands, kidneys, etc. (Coats<sup>16</sup> [case 2]); sepsis due to paratyphoid B infection (Wiechert<sup>12</sup>); gangrenous appendicitis with suppurative peritonitis, suppurative thrombophlebitis, etc. (Pappenheimer<sup>4</sup>); chronic bronchiectasis with pleural empyema (Tilp<sup>17</sup>), and chronic pulmonary and glandular tuberculosis (Liebscher<sup>18</sup>).

In the next two cases, the etiologic factors are chronic infectious toxemias with destruction of bone, and therefore these resemble calcium metastasis. The first (Roth<sup>5</sup>) concerns a 29 year old man who had chronic osteomyelitis of the hand with septicemia, calcium being found in the degenerated muscle fibers in the ventricles and auricles of the heart, in the glomeruli and tubuli of the kidneys and in the interstitial tissue of the mucosa of the stomach. Roth considered the condition to be one of calcium metastasis and probably was correct in the case of the kidneys and the stomach, but the fact that the degenerated muscle fibers were calcified and that the calcium deposits did not have the typical relationship to the blood vessels makes the possibility of a toxic degeneration due to the chronic osteomyelitis more acceptable. The second case described by Siebenmann<sup>11</sup> concerned a 36 year old man

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27. Krayn, M.: Inaugural Dissertation, Heidelberg, 1914.

28. Cutler, E. C., and Sosman, M. C.: *Am. J. Roentgenol.* **12**:312, 1924.

29. Bordenave, M.: *Mém. Acad. roy. d. sc., Paris*, 1768.

30. Simmons, S. F., and Watson, H.: *London Medical Communications*, 1783, p. 228.

31. Burnet, R. W.: *Tr. Path. Soc., London* **32**:53, 1881.

32. Drummond, D.: *Am. J. M. Sc.* **99**:153, 1890.

33. Lucas, J. J. S.: *Brit. M. J.* **2**:1404, 1907.

with chronic advanced osteomyelitis and cystic degeneration of the long bones and chronic nephritis. Extensive calcification was found in the degenerated heart muscle fibers and also in the interstitial tissue, being most abundant about the blood vessels. The lungs, kidneys and liver also showed calcium. This case, again, probably should not be considered one of true calcium metastasis, because the degenerated heart muscle fibers were calcified. A toxic degeneration is more probable.

Included under noninfectious toxemias resulting in calcification of the myocardium are chronic lead poisoning (Langerhans<sup>34</sup>), eclampsia (Roessle<sup>35</sup>), fetal meconium peritonitis (Sturzenegger [case 1]) and bichloride of mercury poisoning (Rüther<sup>36</sup>). The possibility of degeneration of the myocardium due to bichloride of mercury must be kept in mind in Tilp's<sup>37</sup> case, even though the patient died about two and a half days after he had taken the drug.

The records of Rokitansky's,<sup>37</sup> Topham's<sup>38</sup> and the first of Hedinger's<sup>9</sup> cases are so incomplete that they cannot be classified. Though the hyaline degeneration of the muscle fibers in the case in this paper is assumed to have been due to a toxic factor, the cause could not be ascertained.

Finally, Morgagni<sup>39</sup> and von Recklinghausen<sup>40</sup> merely mentioned that they had seen cases of calcification of the myocardium, while the reports of Allen,<sup>41</sup> Benjaminovich<sup>42</sup> and Waldorp<sup>43</sup> were unobtainable, and Oppenheimer's<sup>44</sup> case showed only endocardial changes.

#### SUMMARY

In a 26 weeks old, premature infant who lived for thirty minutes after birth, extensive calcification of the myocardium was found, which is explained as secondary to toxic degeneration of the muscle fibers. A review of the literature shows that calcification of the myocardium is secondary to preceding degeneration or necrosis of the muscle fibers. This degeneration may be due to vascular, inflammatory or toxic conditions. Fatty degeneration plays no part in calcification of the muscle fibers.

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34. Langerhans: *Grundriss der allgemeinen Pathologie und pathologischen Anatomie*, Berlin, 1902, p. 419.

35. Roessle: *Verhandl. d. deutsch. path. Gesellsch.* **10**:303, 1907-1908.

36. Rüther, A.: *Ztschr. f. Kreislaufforsch.* **21**:313, 1929.

37. Rokitansky, C.: *Ztschr. d. k. k. Gesellsch. d. Aertz in Wien* **1**:1, 1849.

38. Topham: *Brit. M. J.* **2**:953, 1906.

39. Morgagni: *De sedibus et causis morborum, Venetiis, ex typog. Remondiniana* 1762, epist. 27, art. 16.

40. von Recklinghausen: *Handbuch der allgemeinen Pathologie des Kreislaufes und der Ernährung*, Stuttgart, Ferdinand Enke, 1883.

41. Allen: *Australian M. J.* **6**:69, 1884.

42. Benjaminovich: *Sibirsk.-Vrach.* **2**:139, 1915.

43. Waldorp, C. P.: *Rev. Asoc. méd. argent.* **37**:74, 1924.

44. Oppenheimer, B. S.: *Proc. New York Path. Soc.* **12**:213, 1912.

# ACUTE PYEMIC CHOLECYSTITIS

REPORT OF A CASE

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Pronounced hematogenous infection of the gallbladder in pyemia is exceptionally rare. Not one such occurrence was noted in 12,000 post-mortem examinations at the German Pathological Institute, Prague, during the past twelve years, despite the fact that among these necropsies were many cases of pyemia. Kaufmann<sup>1</sup> reported briefly two examples of septicopyemia in which at autopsy a fair number of pustules were found in the mucosa of the gallbladder. These changes possessed no clinical significance. Except for Kaufmann's observations, the literature, so far as we have been able to determine, reveals no similar instances, completely described.

A recent case of advanced acute cholecystitis with many embolic lesions without stones, in an anatomically typical staphylococcic pyemia arising from acute osteomyelitis, seems worthy of a report. Of significance are its extreme rarity, its unusual gross and microscopic appearances and its clinical importance.

## REPORT OF CASE

*Clinical History.*—A married white woman, aged 58, was admitted to the Buffalo General Hospital, in the service of Dr. Nelson G. Russell, Sept. 17, 1931, because of pain in the right hypochondrium and back. She died the next day. The family history was unimportant. Four years before her entry into the hospital, she had suffered from a condition which was diagnosed as diabetes mellitus.

The illness in question began a little over a week before admission with a moderately severe pain in the lower part of the back and a slight fever. Minimizing her complaints to the family physician who saw her at this time, she continued up and about, working. Two days later, however, she was forced to bed with a severe pain in the right hypochondrium and fever. Nausea and emesis occurred several times. Acute cholecystitis now suggested itself to her physician. Subsequently her condition became more serious; the abdominal pain was not

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1. Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte, ed. 9-10, Berlin, W. de Gruyter & Co., 1931, vol. 1, p. 929.

relieved, while the pain in the back radiated downward to the thighs and increased to such a degree that she was unable to lie flat in bed; finally semicoma supervened.

The temperature was 99 F.; the pulse rate, 90, and the respirations, 26. The blood pressure was 110 systolic and 72 diastolic. The skin showed no jaundice. Many râles were heard at the bases of both lungs. "Exquisite tenderness" was elicited in the region of the gallbladder. The right upper quadrant was held tense, but not rigid.

The laboratory studies showed diabetes mellitus of moderate severity without significant acidosis, and polymorphonuclear leukocytosis. A blood culture gave six colonies of *Staphylococcus aureus* per cubic centimeter.

*Anatomic Diagnosis.*—The autopsy was made one hour after death. The anatomic diagnosis was: acute, purulent osteomyelitis and periostitis of the body of the third and fourth lumbar vertebrae, extending on the right side into the iliopsoas muscle, with purulent myositis; acute lymphadenitis of the peri-aortic nodes;



Fig. 1.—External view of gallbladder.

staphylococcic pyemia; multiple pyemic lesions of embolic nature in the wall of the gallbladder, with erosions and ulcers of the mucous membrane, and extension outward to the serosa with the formation of acute purulent pericholecystitis and right subdiaphragmatic abscess; pyemic, hemorrhagic infarcts in both lungs, with fibrinous pleuritis; multiple pyemic abscesses of both kidneys; slightly enlarged, somewhat soft spleen; diffuse colloid hyperplasia of the thyroid.

*Gallbladder.*—When the peritoneal cavity was opened, about 200 cc. of bile-stained, purulent, odorless material escaped from the area beneath the costal arch on the right side. The gallbladder seemed somewhat distended and was bound to the colon by recently formed fibrinous shreds. Its shape and position were entirely normal. On the external free surface (fig. 1) appeared a large, flat, pouchlike protrusion of the wall, like a dumb-bell in shape, green, and covered with a thin, fibrinopurulent exudate. Near the neck of the gallbladder were three other yellowish-green patches, from 0.4 to 0.7 cm. in diameter. In addition, the external



free surface was marked with several smaller round, greenish patches, apparently extensions from focal lesions in the wall.

On incision, a fair amount of greenish, mucoid bile escaped. The open gallbladder showed an interesting picture (fig. 2). No stones were present. The mucosa was spotted with rather numerous ulcers and erosions of various sizes with chrome yellow centers, corresponding to the patches noted externally, the circumferences being stained distinctly green from bile imbibition. Two ulcers on the internal free surface, one measuring 2 by 1 cm., the other 1.4 by 1.6 cm., almost ran together, in the dumb-bell-shaped manner indicated. A few pea-sized erosions were noted on the internal free surface throughout the region of the neck. Similar medium-sized erosions were scattered on the internal upper surface. Between these large and medium-sized ulcers and erosions were sprinkled small erosions of about pencil-point size and dark green. The mucosa otherwise showed

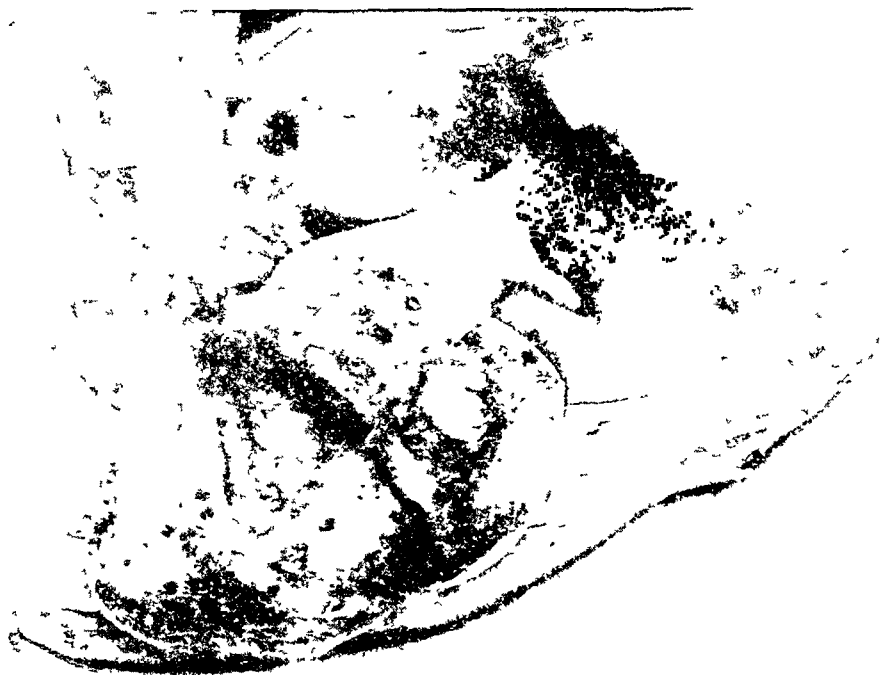


Fig. 2.—Internal view of gallbladder.

distinct injection. No real abscesses and no small pustules were present. The wall showed no appreciable thickening, in its thickest portion it measured 0.2 cm. The intrahepatic, hepatic, cystic and common bile ducts were not dilated. Their mucosa was not injected, and the bile which they contained was normal in appearance. The cystic duct node was completely surrounded by fat and apparently not swollen. The portal vein and hepatic arteries were normal. The pus that exuded when the abdomen was first opened came from a large abscess bounded by the diaphragm, parietal wall, liver, colon and right kidney, the upper pole of which was 5 cm. lower than that of the kidney of the other side. The liver was slightly atrophic and rather soft. In many cross-sections no abscesses were observed.

The pancreas weighed 25 Gm.

*Bacteriology.*—Smears from the bone and periosteum of the third and fourth lumbar vertebrae, iliopsoas muscle, subdiaphragmatic abscess, kidneys, wall of the gallbladder, bile in the gallbladder and hepatic and cystic ducts revealed an unusually large number of staphylococci. No other micro-organisms were seen.

*Microscopic Examination.*—Sections of the gallbladder were made from different parts, including the large deep ulcers, superficial erosions, neck and uninvolved areas. The cystic duct was also examined. Hematoxylin-eosin and Gram-Weigert bacterial stains were used. The histologic picture of the different embolic lesions, erosions and ulcers, noted grossly, was clear and similar in all the sections examined, but rather unusual. Throughout, it was characterized primarily and uniformly by the presence within capillary vessels, as well as in the wall of the gallbladder adjacent to them, of large bacterial masses with marked necrosis and extensive recent hemorrhage of the surrounding tissue. The changes in the necrotic tissue resembled definite autolysis, which was confined to the vicinity of the bacterial masses. The different histologic lesions tended to be trapezoid or wedge-shaped. Forming the borders of a few necrotic foci many leukocytes were observed, but principally near a majority of the embolic necroses there was almost complete absence of severe reactive cellular infiltration. Rather surprising was this unusual general lack of distinct abscess formation. In the wall forming the margin of bacterial masses that could be traced in the deep layers—for instance, in the bases of small ulcers and recent erosions—we found only a moderate number of lympho-



Fig. 3.—Huge focus with marked necrosis involving all layers of the wall extending to the serosa, and with large bacterial masses on its margin. The mucosa is destroyed. The wall adjacent to the focus is relatively unaffected; the mucosa here is still preserved, and the cellular reaction is minimal; low power magnification.

cytes and a distinct proliferation of young mesenchymal cells, the remainder of the adjacent wall not being remarkable. At the bases of large ulcers, a few leukocytes with inflammatory edema were occasionally seen. In some sections, especially in the large subserous abscess-like foci, the serosa, which was in most parts only distended and free from surface exudate, showed, along with the marked inflammatory edema, a rather pronounced leukocytic, almost phlegmonous inflammation and fibrinous exudation. Much of the mucosa on the surfaces of recent superficial lesions was preserved, overhanging, almost covering the whole area of each embolic focus; the mucosa opposite the inner surfaces of deep foci was usually entirely normal.

Examination of those parts of the gallbladder in which grossly no erosions or embolic foci were seen, and of the cystic duct, showed only injection of the veins and slight edema of the subserous tissue. Especially noteworthy was the absence of any fibrosis or signs of rebuilding of the wall, such as are found in chronic cholecystitis. The mucosa was not changed.

In many sections of the liver no abscess formations were seen.

The right iliopsoas muscle showed dissecting interstitial, almost phlegmonous inflammation with very large abscess formation and necrosis of the muscle fibers.

In the peri-aortic nodes was found inflammatory hyperplasia with leukocytic exudate into the sinuses with marked swelling of the reticulum cells.

The kidneys disclosed typical excretory abscesses in an early stage with bacterial masses in the centers.

In the tail and body of the pancreas the islands of Langerhans were decreased in number.

The lungs showed septic hemorrhagic infarctions in rather early stages.

#### COMMENT

This case seems explained satisfactorily by the occurrence of primary acute osteomyelitis of the lumbar vertebrae and staphylococcic pyemia, followed soon after by acute pyemic cholecystitis, which completely dominated the clinical picture.

The anatomic and histologic findings make it clear that in the wall of the gallbladder were multiple severe pyemic lesions of embolic nature, characterized by the presence of large bacterial masses and severe necrosis, but by the absence of abscess formation, extending to the serosa, and thence causing, without a distinct gross perforation, acute focal peritonitis.

The lesions in the wall of the gallbladder can be accounted for only on a hematogenous basis. The condition was one of typical pyemia with a positive blood culture of *S. aureus*. The histologic changes began in the deeper layers of the wall, the ulcers and erosions occurring secondarily. They consisted fundamentally of multiple bacterial emboli in capillary vessels with invasion of the surrounding tissue by bacteria, and with involvement of the invaded tissue by necrosis and extensive hemorrhage.

The presence of bacteria in the bile can almost certainly be explained by direct passage of micro-organisms into the lumen from perforating lesions in the deeper layers of the wall of the gallbladder, rather than by excretion of those organisms with the bile from the liver into the ducts, with subsequent descending infection of the gallbladder. In many sections of the liver, no abscesses or areas of necrosis were found. There were no stones or occlusions of the cystic or common bile ducts, and in the absence of such factors it has proved until now impossible, by experimental introduction of virulent bacteria into the gallbladder, to produce cholecystitis. The erosions in different areas of the gallbladder resembled in no way decubitus ulcers caused by stones. It is perhaps interesting to note here that the bile in two other cases of pyemia in which autopsies were made recently, one due to acute staphylococcic osteomyelitis of the left femur and the other to strep-

tococcic infection of the upper respiratory tract, proved to be sterile on culture. That the gallbladder had been the seat of previous recurrent inflammatory attacks is improbable, because no thickening of the wall, no fibrosis of the subserosa and no scar formation in the mucosa were demonstrable.

An analysis of the histologic picture leads to the conclusion that the changes produced by the unusually large bacterial emboli must be considered as infarcts or anemic necroses, rather than as real abscesses. The marked necrosis with severe hemorrhage around the bacterial masses, and in a few instances with an additional outer border of marked leukocytic infiltration, together usually forming a wedge-shaped focus, supports from a structural point of view the conception of a process of acute embolic necrosis or infarction. No real abscesses or pustules, such as Kaufmann described, were seen in this case. Yet the lesions in the gallbladder, in view of the clinical history, could hardly have been antedated by those in the kidneys, which were typical pyemic abscesses. We can only speculate whether some lesions had already extruded their purulent content into the lumen of the gallbladder by perforation through the mucous membrane, or whether leukocytic infiltration had no favorable opportunity to appear in the face of an overwhelming infection progressing rapidly with marked necrosis through the loose tissue in the thin-walled organ.

The localized peritonitis and subdiaphragmatic abscess, in the absence of a demonstrable gross perforation, probably occurred by penetration of organisms at the site of the dumb-bell-shaped serosal protrusion. We believe that at these points there was rapid penetration through large sections of excessive necrosis within the wall.

#### SUMMARY

The clinical record and pathologic findings in an unusual case of advanced acute pyemic cholecystitis with multiple embolic lesions in the wall of the gallbladder, which occurred during staphylococcic pyemia arising from acute osteomyelitis of the lumbar vertebrae, are reported. No similar observation appears in the literature. Grossly, the internal surface of the gallbladder showed superficial erosions and ulcers; externally, without a gross perforation, the formation of acute pericholecystitis and right subdiaphragmatic abscess had taken place. No real abscesses were found in the wall of the gallbladder. Histologically, the lesions observed in the wall were best characterized as acute infarctions from bacterial emboli. Clinically, the symptoms referable to the pathologic changes in the gallbladder tended to obscure all other manifestations of the primary disease with which the patient, an elderly diabetic woman, was suffering.

# VASCULAR LESIONS OF THE GASTRO-INTESTINAL TRACT IN MERCURY POISONING

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On Dec. 11, 1927, a patient who had suffered for nine days with acute abdominal pain, associated with chills, fever and night sweats, was examined post mortem. The examination revealed 25 cm. of gangrenous ileum which had become completely separated from the canal by sharp lines of demarcation. The mesentery leading to this segment of intestine was markedly thickened and indurated. The rest of the gastro-intestinal tract was normal. It was obvious that there was marked vascular injury in this part of the mesentery. The cause of the injury was not apparent at the time, but we are inclined, as a result of subsequent studies, to ascribe it to the ingestion of mercuric chloride administered in the course of antisyphilitic treatment.

On Nov. 26, 1929, another patient who had been ill with diarrhea for two years, and who recently had exhibited evidence of peritonitis, was examined post mortem. The examination established that the terminal 3 feet (92 cm.) of ileum was ulcerated, and that the mesentery was much thickened. There were perforations at the ileomesenteric angle. This, again, indicated nutritional disturbance of a limited portion of the intestinal wall. According to the patient's history, a persistent diarrhea followed immediately on the taking of thirty doses of mild mercuric chloride at intervals of thirty minutes. The pathologic changes suggested the case noted in the preceding paragraph. As it was realized that mercury might cause the extensive vascular lesions of the order found, 517 Gm. of involved mesentery and intestine was chemically analyzed for mercury, and 3.88 mg. of metallic mercury was recovered.

In a third instance, in the fall of 1931, the ulcerated stomach and thickened, constricted large bowel at postmortem examination were sent to the department for histologic examination. Lesions were revealed which were similar, but more active, than those in our second case in

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which mercury had been found. Mercury poisoning was suspected, and an examination for mercury was made; 317 Gm. of this tissue yielded 12.1 mg. of mercury.

According to present knowledge, the amounts of mercury found in these tissues are distinctly abnormal, since only on occasions are traces of mercury found in the tissues of patients who have amalgam fillings (Schreiber in personal communication). We believe that mercury could have caused the vascular lesions noted. In view of the similarity of the lesions in these three cases and the finding of significant quantities of mercury in the tissues in the two cases in which chemical examinations for mercury were made, the concurrence would seem to indicate a highly probable relationship.

#### REPORT OF CASES

CASE 1.—A white switchman, aged 44, who entered the University Hospital on July 6, 1927, had a draining perirectal fistula for four years, dizzy spells for three years and nervousness for two years. His past diseases included, besides the ordinary diseases of childhood, scarlet fever at 3, malaria at 14, pneumonia at 32 and 37, diphtheria at 41, gonorrhea four times and a primary penile lesion at 22 years of age. His recovery from these diseases was apparently uneventful, except for an occasional attack of painless jaundice following the malaria. At the age of 38, he had an attack during which he complained of gas in the stomach and an ache in the abdomen. This was accompanied by jaundice and clay-colored stools. He was treated at a "resort" for six weeks, where considerable mild mercuric chloride was administered (the exact amount could not be ascertained). That same year he suffered with diarrhea for five weeks, during which he noted blood and yellowish mucus in his stools. Four years prior to admission, he had an ischiorectal abscess, which was drained. A persistent draining fistula remained. His dizzy spells were not serious.

Physical examination showed nothing abnormal other than the ischiorectal fistula and slightly thickened peripheral arteries. The Wassermann reaction of the blood was positive; otherwise there were no laboratory findings of significance.

After the administration of six 0.9 Gm. doses of neoarsphenamine, an excision of the perirectal fistulous tract was performed. The recovery was uneventful. He was discharged on Aug. 18, 1927, and during the months of October and November he received two doses of one thirty-second grain (0.002 Gm.) of mercuric chloride by mouth every other day. He returned to the hospital on Dec. 2, 1927, complaining of severe pain in the lower left abdominal quadrant and of nocturnal sweats and chills. This illness began on November 20, with severe cramping pain in the left side of the abdomen. Physical examination revealed an exquisitely tender mass in the left lower quadrant of the abdomen. The urine contained a slight trace of albumin, but no blood. Perforated carcinoma of the sigmoid was diagnosed at operation on Dec. 6, 1927. Blood was passed in the stools following operation. Death occurred five days later, twenty-one days after the symptoms began.

*Autopsy.*—Postmortem examination revealed a well nourished, markedly jaundiced man with no evidence of edema. Investigation of the abdominal contents revealed a large cavity beneath the operative incision, the walls of which were formed by the great omentum, the anterior parietes and a loop of small intestine.

These were agglutinated one to another by fibrinous adhesions. The cavity contained a dirty, fluid material. The loop of intestine involved in the cavity was found to be 25 cm. of the lower ileum. This segment of intestine was completely gangrenous. Its proximal and distal portions had become completely separated by rather sharp lines of demarcation from the canal, so that the lumina of both ends communicated freely with the cavity. The mesentery leading to this segment of intestine was markedly thickened and indurated. The entire intestinal canal below this point contained blood. The remaining gross findings were those of profound sepsis. No neoplasm was found.

*Histologic Observations.*—Microscopic examination of the mesentery leading to the gangrenous segment of bowel revealed very marked obliterative arteritis, involving the majority of the medium-sized vessels. Some of the vessels were occluded by organized thrombi, while others showed marked subintimal connective tissue proliferation resulting in slitlike lumina. Some of the latter vessels were occluded by fibrinous thrombi. The lumina of many small vessels contained fibrinous and hyaline thrombi. Subintimal connective tissue proliferation was present in several of the large vessels, but no occlusion was demonstrated. The supporting fat tissue was markedly and diffusely infiltrated with acute inflammatory cells. A thick layer of fibrin was deposited on the peritoneal surfaces. The liver presented the picture of early diffuse toxic necrosis. The kidneys showed no evidence of acute nephritis. The histologic findings in the remaining organs were those of profound sepsis. A streptococcus was cultured from the mesenteric lymph nodes.

CASE 2.—A white plasterer, aged 65, entered the University Hospital on July 9, 1929, complaining of diarrhea, soreness in the right side of the chest and a hacking cough. In July, 1928, he took thirty consecutive doses of mild mercuric chloride at intervals of thirty minutes for an attack of "influenza." A persistent diarrhea of from six to twenty stools a day followed. He had never noticed mucus, pus or blood in his stools. His diet was apparently sufficient prior to his illness. In May, 1929, pigmentation, dryness and scaling of the hands appeared, and at this time a dry cough developed, accompanied by pain in the right side of the chest. His loss in weight was 46 pounds (20.8 Kg.). He stated that his father, mother and maternal grandparents died of diarrhea, but that none of them had dementia or dermatitis.

Physical examination revealed an irritable, emaciated man, whose skin was dry and scaly. Other positive findings were unequal pupils, which reacted 50 per cent to light, tortuous and hardened peripheral arteries, some rigidity of both recti muscles and slight tenderness in the right lower abdominal quadrant. Laboratory examinations revealed an absence of free hydrochloric acid in the stomach and the presence of occult blood and undigested food in the stools. The Wassermann reaction of the blood was negative. The patient was discharged on Aug. 10, 1929, with a tentative diagnosis of pellagra. Because his condition did not improve, he was readmitted on Sept. 14, 1929. Reexamination revealed secondary anemia, an increase in the loss of weight to 78 pounds (35.4 Kg.) and roentgen evidence of mucous colitis. He was discharged unimproved on Oct. 5, 1929. On Nov. 16, 1929, he was again admitted because his symptoms had gradually become more pronounced. The physical findings suggested an acute abdominal condition, which was thought to be peritonitis due to a perforated carcinomatous ulcer of the colon. Death occurred on Nov. 25, 1929, sixteen months after the ingestion of mercury.

*Autopsy.*—Postmortem examination revealed an emaciated man, whose skin over the knees, extensor surfaces of the arms, forearms and back was rippled, scaly and purplish. Brownish pigmentation of the skin was limited to the back of the hands. The peritoneal cavity contained 250 cc. of pus and intestinal content.

Fibrin covered the peritoneum. The cecum, the distal loops of the ileum and the sigmoid colon were densely adherent to each other by fibrous adhesions. Several pockets of pus were discovered between the loops of intestine. After the intestinal canal was thoroughly exposed, it became obvious that the important lesion was in the terminal 3 feet (92 cm.) of the ileum. Marked ulceration of the mucosa of this portion was observed. The ulcerated areas increased in number and size from above downward, until practically the entire mucosa was involved at the

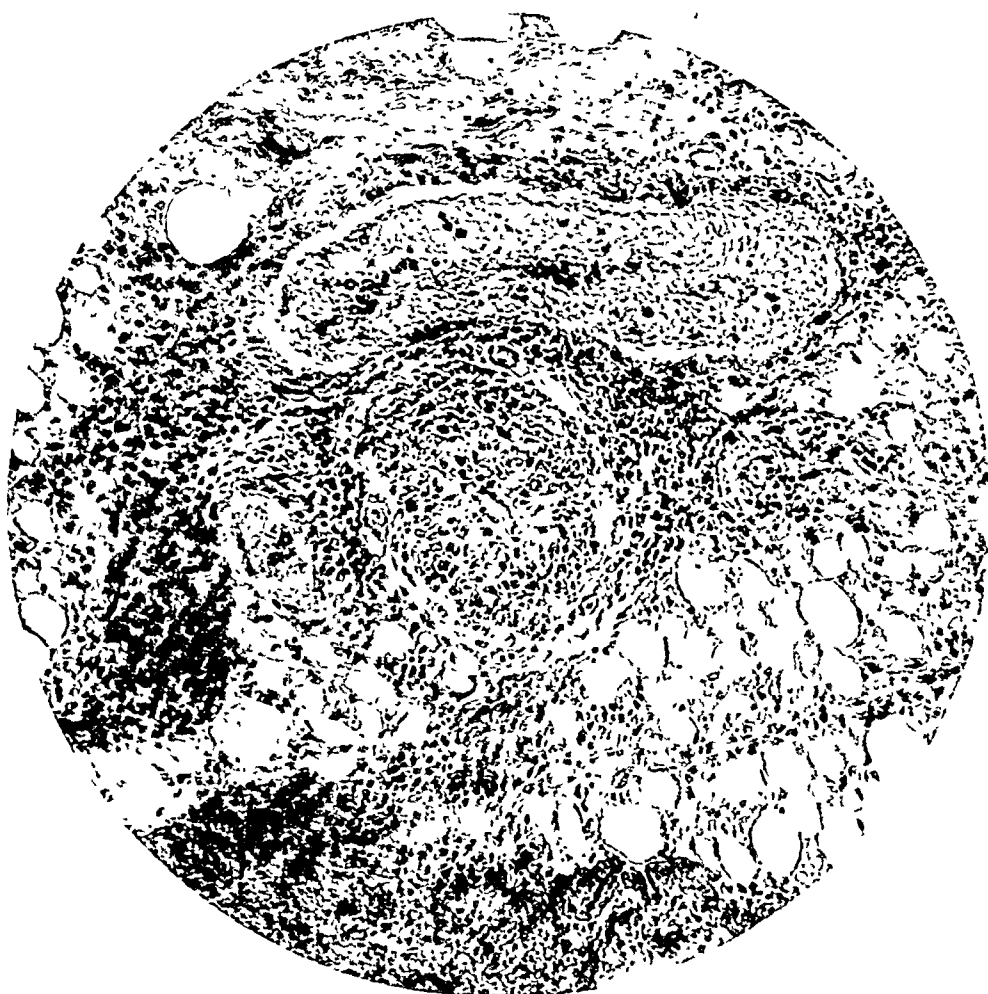


Fig. 1 (case 2).—Thickened veins and occluded arteries in mesentery;  $\times 160$ .

ileocecal junction. The wall was extremely firm, measuring 1 cm. in thickness. At the ileomesenteric angle, several perforations were found. The mesentery leading to this portion of the intestine was thick and firm, and the thickest portion was definitely associated with the most extensive changes in the wall of the intestine. No ulcerations were found in the remainder of the gastro-intestinal tract. Additional findings were septic splenitis, bronchopneumonia and brown atrophy of the heart.

*Histologic Observations.*—Microscopic examination of a transverse section of mesentery leading to the ulcerated intestine revealed marked obliterative arteritis. Practically all of the medium-sized and small vessels showed extensive subintimal



connective tissue proliferation with reduction of their lumina to small slits. Some of the vessels were completely occluded, either by organized or by hyaline thrombi. Hyalinized fibrin was noted in the walls of some of the vessels. There were considerable subintimal deposits of connective tissue in the large vessels, but none were occluded. A collar of lymphocytes was present around some of the vessels. Many lymphocytes and endothelial leukocytes and a few polymorphonuclear leukocytes had infiltrated the supporting scarred fat. The intestinal wall was thickened

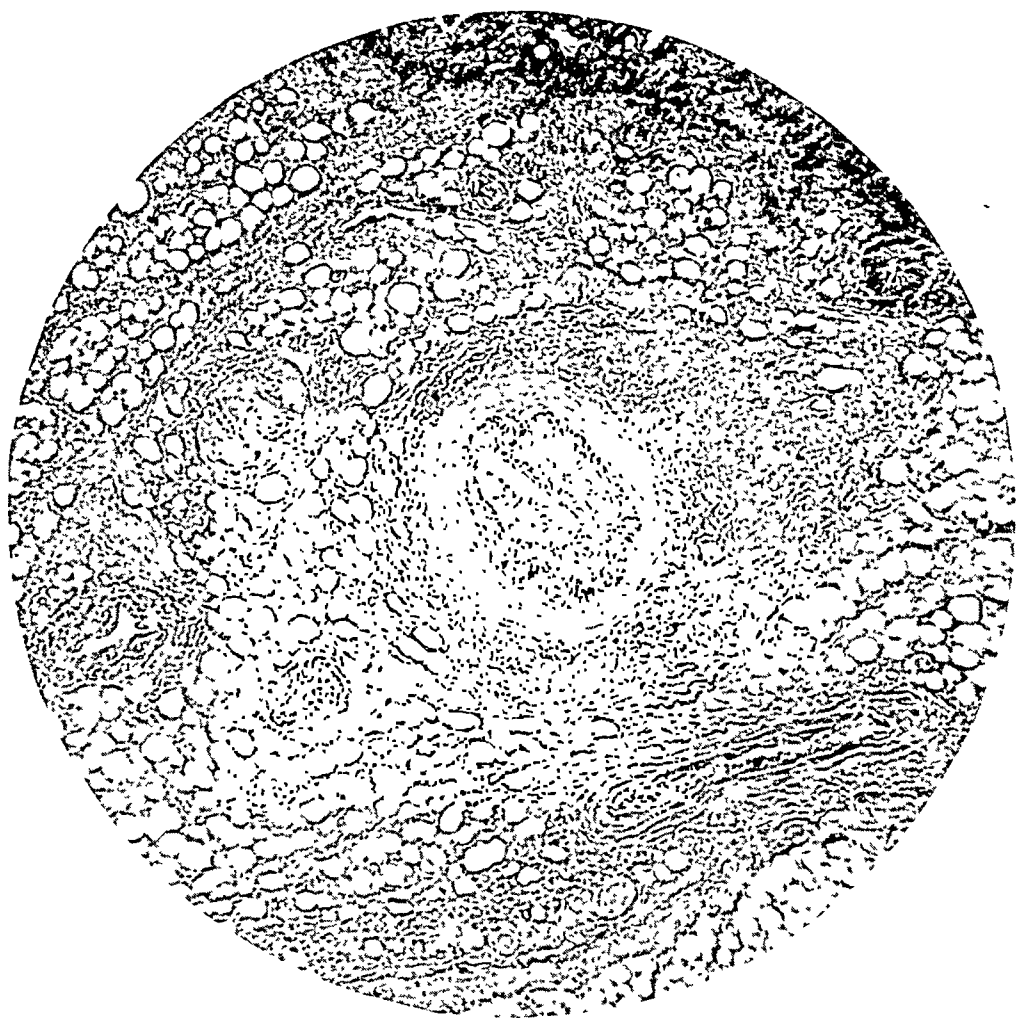


Fig. 2 (case 2).—Marked endarteritis in the vessels of the mesentery;  $\times 160$

because of marked scarring. Granulation tissue, heavily infiltrated with chronic inflammatory cells, covered the denuded mucosal surfaces. The histologic changes in the remaining organs were those resulting from sepsis.

Five hundred and seventeen grams of tissue was examined for mercury. The content of mercury, expressed as metallic mercury, was 3.88 mg.; 0.53 mg. was found in 83 Gm. of mesentery, and 3.35 mg., in 434 Gm. of intestine.

CASE 3.—A white woman, aged 68, whose past history was unavailable, was seen in a moribund state by her physician in March, 1931. No history of her acute illness was obtainable. Physical examination revealed a profoundly ill, dehydrated, somewhat emaciated woman with a markedly distended abdomen. Red

blood was noted in both the stool and the vomitus. Attempts at giving enemas failed. Acute intestinal obstruction was diagnosed, but any operative procedure was deemed not advisable. Death occurred four days after the woman was first seen.

*Autopsy.*—Postmortem examination was limited to the contents of the abdomen. The pyloric end of the stomach and the terminal 6 feet (183 cm.) of ileum, with the cecum and a portion of the descending colon, were sent to the pathologic laboratory for study.

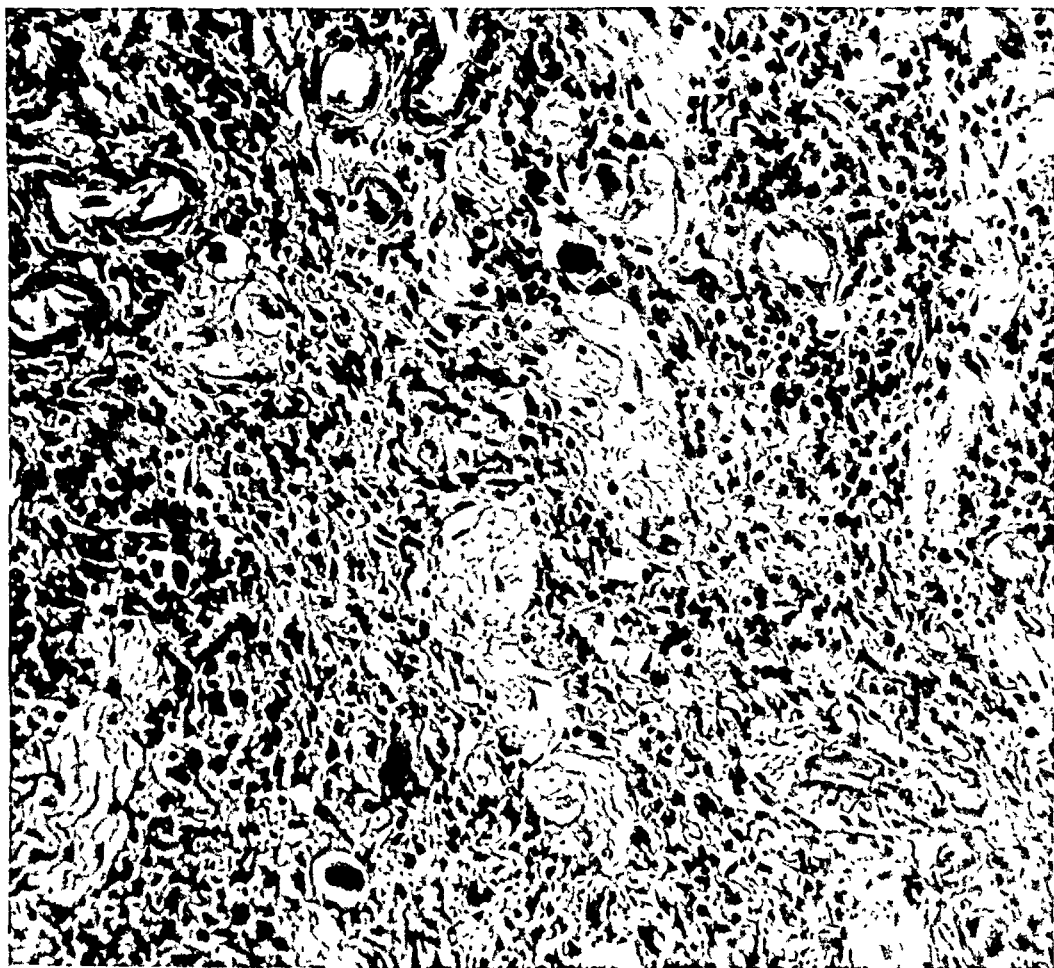


Fig. 3 (case 2).—Tissue from intestinal wall;  $\times 220$ . Note fibrosis of small vessels, hyaline thrombi and atypical granulation tissue.

Examination of the stomach showed a superficial, somewhat irregular ulceration near the pylorus. The upper portion of the ileum appeared normal, but superficial diffuse ulceration of the mucosa of the lower portion was in evidence. Marked constriction of the colon was present, the total diameter being only 1.2 cm. The wall was extremely firm. Its entire mucosa was replaced by a homogeneous gray, firm, friable membrane.

*Histologic Observations.*—Microscopic section of the gastric ulcer revealed a denuded area of mucosa replaced by granulation tissue covered by fibrin. Polymorphonuclear leukocytes infiltrated the ulcer bed and underlying gastric wall. A

superficial portion of the mucosa of the lower part of the ileum that was necrotic and covered by fibrin heavily infiltrated with polymorphonuclear leukocytes presented the only histologic change in the ileum. Evidence of extreme injury was present in the colon. Practically the entire mucosa was necrotic. Many arteries in the submucosa contained fibrinous thrombi in variable amounts from those causing complete occlusion to small intimal deposits. The walls of many of the blood vessels consisted merely of irregular strands of homogeneous pink-staining tissue infiltrated with polymorphonuclear leukocytes. Hyaline thrombi were present

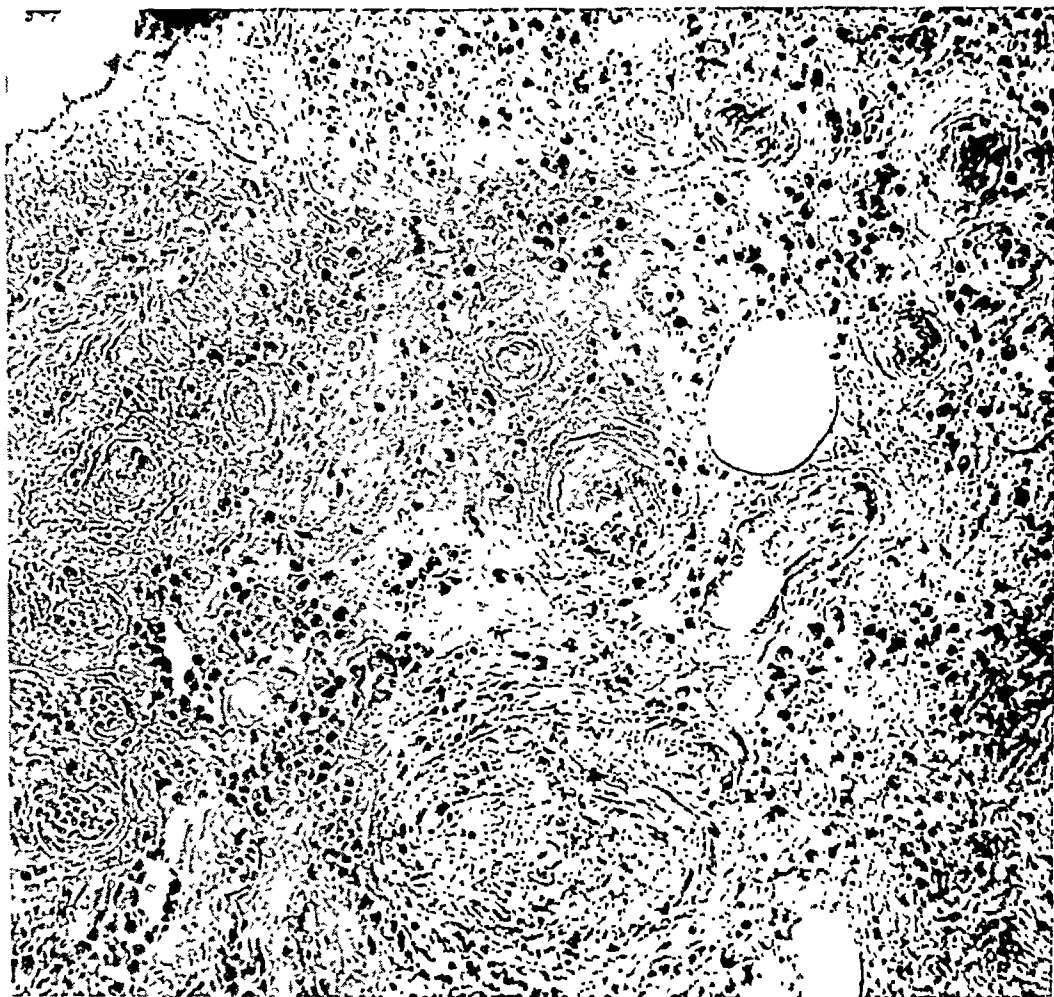


Fig. 4 (case 3).—Colon tissue;  $\times 220$ . Note rather acute injury of vessels and thrombosis, as well as acute inflammation.

in many very small vessels. Edema was marked throughout the entire wall, with a diffuse infiltration by acute inflammatory cells.

#### COMMENT

The mercury was isolated as the metal by Schreiber, according to the method evolved by Booth, Schreiber and Zwick for the determination of mercury in the presence of organic matter. In the second case, 517 Gm. of intestine and mesentery contained 3.88 mg. of mercury.

The mesentery removed, so far as this was possible, weighed 83 Gm. and yielded 0.53 mg. of mercury. The 434 Gm. of intestines yielded 3.35 mg. of mercury. The concentrations of mercury in the mesentery and intestine were 0.65 and 0.77 mg. of mercury per hundred grams, respectively. In the third case, the mercury found in 317 Gm. of tissue was 12.1 mg. The 42 Gm. of colon contained 9.9 mg.; the 45 Gm. of stomach, 0.2 mg., and the 230 Gm. of ileum, 2 mg. The concentrations in these various tissues were 23.5 mg., 0.44 mg. and 0.87 mg. per hundred grams of colon, stomach and ileum, respectively. The concentrations of the mercury in the tissues in the two cases in which mercury analyses were made paralleled the extent and the acuteness of the lesion described, with the exception of the mercury content of the gastric tissue, which was examined only in one case, and which showed a lower concentration of mercury than the other tissues in either case.

The question of whether mercury, like certain of the other metals, is a normal constituent of tissue may readily be raised. The literature

TABLE 1.—*Results of Analysis of Tissues for Mercury (Case 3)*

Organ	Weight, Gm.	Metallic Mercury, Mg.
Colon.....	42	9.9
Stomach.....	45	0.2
Ileum.....	230	2.0
Total.....	317	12.1

does not reveal much on this point. Traces of mercury are, at times, found in the tissues of persons who have had some of their teeth filled with amalgam. In two of our cases, mercury in some form was known to have been ingested. Schreiber stated that examination of tissues in cases in which it was certain that mercury had not been taken yielded no mercury.

The lesions produced in all three cases seemed to be primarily injuries to the walls of blood vessels—more prominent in arteries and small arterioles than in veins. There was proliferative endarteritis as well as thrombo-arteritis in the more acute cases. The extent of these changes was such that the intestinal walls had suffered markedly from ischemia.

One of the patients in our cases had chronic diarrhea. All three cases were eventually diagnosed carcinoma with perforation because of chronic intestinal obstruction and evidence of peritonitis.

It is striking that stomatitis and acute tubular nephritis, the lesions that we are wont to associate with mercury poisoning, are not integral parts of the clinical manifestations of this particular type of mercury poisoning. The lesions are primarily vascular. A limited portion, and not always the same portion, of the gastro-intestinal tract in the three

cases under consideration was diseased. This would imply the involvement of a limited portion of the vascular tree of a different part of the gastro-intestinal tract in each instance. The finding of mercury in tissues sixteen months after the ingestion has impressed us with the length of time during which this injurious agent may act. It will be interesting to see whether these divergent and disturbing features of the disease can be brought into accord by what is known of mercury and the reaction it causes in tissues.

The fate of mercury, when injected into the body or taken by mouth, is in part well known. The excretion by the way of the kidneys, the stomach, the mouth and the intestines with resultant lesions is well known in the more acute cases of mercury poisoning. We have learned from past experiences that whenever a new mercury compound is suggested for the relief of edema or as a genito-urinary antiseptic, and so on, the patient must be kept under observation for evidences of

TABLE 2.—*Circulation of Mercury: Amount of Mercury per Hundred Grams of Tissue After Intramuscular Injection of Various Compounds as Indicated*

Tissue	Mercurium Benzoicum, 37.8 Mg.	Acidum Mercuric Salicylicum, 33.5 Mg.	Mild Mercuric Chloride, 48.0 Mg.	Metallic Hg (50% Oil Suspension), 154 Mg.
Liver.....	0.49	1.16	0.37	0.65
Kidney.....	2.20	5.03	2.12	1.75
Small intestine and feces.....	0.39	0.55	0.19	0.29
Large intestine and feces....	0.32	0.53	0.20	0.13

irritation of the kidneys, such as albumin and blood in the urine, or for gastro-intestinal injury evidenced by diarrhea and blood in the stool. The distribution of various mercury compounds in the tissues, which concentrate them energetically, may be noted in table 2, which was compiled from the work of Lomholt.

Cole, Gammel, Schreiber and Sollmann, as well as Lomholt and others, have found that after a short period of active elimination, the excreted mercury being only a small percentage of that injected, elimination falls rapidly to traces, which may persist day after day. Cole and his co-workers stated that a week after the injection of mercury salicylate, from 85 to 90 per cent remains unexcreted. With these facts before us, it is not difficult to believe that mercury may be found in the intestinal wall, which represents one of the concentration points of mercury. several years after ingestion.

Authors are well in accord that the gastro-intestinal tract is second only to the urinary tract in the excretion of mercury compounds. Cole and his co-workers stated that with mercury salicylate the excretion in the feces is about one-twelfth that of the urinary elimination. The literature in general is also quite clear that the excretion occurs along the

entire length of the gastro-intestinal tract. Bargaen, Osterberg and Mann, however, found, in contrast with the general experience, that the colon of a dog had nothing to do with absorption and excretion of the mercury compounds that they studied. The opinion that the important reason for the concentration of mercury in the kidneys, liver and intestinal wall is for the purpose of excretion may be altered by this bit of evidence.

Another probable reason for the concentration of mercury along the gastro-intestinal tract was pointed out by Almkvist. He noted yellowish-black particulate matter in leukocytes and free in tissue in cases of mercury poisoning. He also found experimentally that hydrogen sulphide would precipitate the mercury in the tissues of the gastro-intestinal tract to the extent that yellowish flakes could be observed in the gross specimen. The fact has been pointed out that yellowish-black granules are found in such locations as the kidneys, where hydrogen sulphide probably does not play an important part in their formation. Elbe believed that the granules noted by Almkvist may be altered blood. The accumulation of mercury along the intestinal wall for excretion would seem to imply that the concentration should be regularly greater in that part of the gastro-intestinal tract more actively concerned with the excretory function in question. At this location, one would logically and regularly expect the more extensive lesions. This has not been true. Concentration of mercury between the large bowel and small bowel has varied as indicated in table 2. In our cases, the severe lesions were in the colon in one instance, while in the other two instances the lesions were confined wholly to the ileum. This would call for a factor in addition to that of concentration of the poison and injury to the tissue at the point of excretion. Almkvist's explanation would satisfy as to this variability of the amount of mercury and of the severity of the lesions along the gastro-intestinal tract. The precipitation of mercury and the extent of the lesion in a given case would be greatest at the point along the gastro-intestinal tract where the concentration of hydrogen sulphide chanced to be the more abundant at the time the mercury happened to be circulating in the blood.

It seems pertinent to consider the presence of gastro-intestinal lesions in the absence of tubular nephritis and stomatitis due to mercury poisoning. The fact that mercury may be excreted in the urine without renal lesion is well known. Rosenheim noted in his perfusion experiments that weak solutions of mercury compounds caused vasoconstriction, while stronger solutions not only caused vasoconstriction, but irritated the renal parenchyma and caused diuresis. On the other hand, patients receiving mercury therapy frequently have diarrhea and

even blood in the stool in the absence of stomatitis or nephritis. Again, whether Almkvist's interpretation of his yellowish-black granules is the correct one or not, there seems to be a factor that operates along the gastro-intestinal tract rendering the concentration of mercury in mercury poisoning largely a matter of chance, in the more chronic types of mercury poisoning. It would be possible, under such conditions, to obtain high concentrations of mercury in various parts of the tract without either nephritis or stomatitis.

Information on what happens and on the sequence of events in the production of the lesion can be gathered from the literature. First, the poison must be concentrated in a certain locality in order to produce a lesion. The corrosive action of heavy metals may be effective at the point of application, where the concentration may be great, or at the point where it accumulates for excretion. Added to this, one has a second factor, which may be the precipitation of mercury pointed out by Almkvist. Niklasson and Santesson indicated, as a result of their investigations, that not only is the presence of mercury in the tissue necessary, but it must be in ionic form to be injurious to tissue. The observations on vasoconstriction made by Rosenheim in his perfusion experiments have also been pointed out by Elbe, Weiler, Natus, Kaufmann and others. The sequence of effects of mercury on vessels and contained blood is: constriction, dilatation, constriction, stagnation and diapedesis of red blood cells. This is virtually an infarction without complete occlusion of the vessel. Kaufmann noted that the stasis resulted in hyaline thrombi in vessels. He believed that the stasis, the hyaline thrombi and the action of bacteria are sufficient to explain the changes found. However, the destruction of intima, the formation of mural thrombi and the injury to the wall of the vessel in our third case are precisely the changes described by Wolff as occurring when he injected mercuric chloride directly into the veins of rabbits. It would seem from the evidence presented that in mercury poisoning there is an action peripherally on the vasomotor mechanism at the point where the mercury is concentrated, plus the irritant action of a foreign body, as well as the corrosive action of an ionizable mercury compound, all acting concurrently on the tissue concerned. Naturally, after the tissue is thus devitalized, the factor of infection is added.

#### SUMMARY

Three cases of a peculiar type of mercury poisoning are described. The lesions were primarily vascular. A limited portion of the gastro-intestinal tract was involved in each case. The same part of the intestinal tract was not involved in any two of the cases. No stomatitis or nephritis was noted.

The peripheral action of mercury on the vasomotor mechanism and the corrosive action of ionizable mercury compounds, as is pointed out in the literature on experimental mercury poisoning, are sufficient, with the additions of the action of mercury as a foreign body and the unavoidable secondary infection, to account for the lesions found.

Chronic intestinal obstruction with evidence of perforation should prompt one to consider the possibility of mercury poisoning.

A postmortem examination that shows primary vascular injury to a limited portion of the gastro-intestinal tract should suggest mercury poisoning as a possible cause until it can be excluded by careful chemical analysis for mercury.



# EXPERIMENTAL PATHOLOGY OF THE LIVER

## VIII. EFFECTS OF CARBON TETRACHLORIDE ON THE NORMAL AND ON THE RESTORED LIVER AFTER PARTIAL HEPATECTOMY

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Since carbon tetrachloride has proved of value as an anthelmintic in clinical medicine,<sup>1</sup> its toxic effects should be thoroughly understood. Experimental studies have shown that the liver is highly susceptible to the drug, and that repeated injections of it may induce cirrhosis.<sup>2</sup> In order to produce marked cirrhosis, however, carbon tetrachloride must be given for a considerable period; with sufficient intervals between injections, regeneration may go on without any permanent changes being induced in the organ. Such regeneration of hepatic parenchyma is probably similar to that which takes place after partial removal of the organ.<sup>3</sup> Regeneration after partial hepatectomy, however, may be repeated indefinitely, whereas in cases of continuous injury to the liver, such as that induced by carbon tetrachloride, complete regeneration finally fails to take place and connective tissue proliferation ultimately produces cirrhosis. In partial destruction of the lobules of the liver by such a toxic agent as carbon tetrachloride, replacement occurs at the site of injury and a new hepatic lobule is regenerated. On the other hand, the growth of the remnant of liver, after partial hepatectomy, is a compensatory hyperplasia in which an attempt is made to restore the original volume of hepatic tissue. The term regeneration as used in this report includes recovery following poisoning by carbon tetrachloride, and the term restoration denotes changes that ensue following partial removal of the organ.

Since restoration of the liver occurs so rapidly following partial removal of the organ, in which no doubt a return to a primitive or an embryonic condition may take place, I wished to know whether the

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From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

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newly restored liver would react more or less intensively to carbon tetrachloride than the normal liver. Accordingly, the investigation was conducted along three lines: (1) the effect of carbon tetrachloride on the normal liver; (2) the effect of carbon tetrachloride during restoration of the liver, when administered prior to partial removal, and (3) the effect of carbon tetrachloride during restoration of the liver, when administered after partial hepatectomy. White rats were used in the study.

#### EXPERIMENTAL METHOD

In most of the experiments 0.25 cc. of carbon tetrachloride was given by stomach tube twice a week. As the effects of different diets on the entire process of hepatic injury have been emphasized,<sup>4</sup> the rats were maintained on an adequate diet consisting of corn meal, linseed oil meal, crude casein, alfalfa meal, salt, calcium carbonate and powdered skim milk.

All operations of partial hepatectomy were performed under aseptic technic and ether anesthesia, and sections were fixed in 10 per cent formaldehyde, stained with hematoxylin and eosin, scarlet red, van Gieson's stain and Mallory's stain for connective tissue. Bile capillaries were stained with the technic of Otani.<sup>5</sup> For the observation of certain cytologic details, especially mitochondria, sections were treated according to the silver and gold method of del Rio Hortega. Other staining methods for mitochondria were occasionally used for comparison.<sup>6</sup>

#### OBSERVATIONS

In order to determine the effect of a single injection of carbon tetrachloride on the liver, 0.25 cc. of the drug was given by mouth to each of a series of rats. At intervals after the administration, a lobe of the liver was removed for examination. Within three hours, the liver appeared to be slightly injured. Besides congestion, the central areas of the lobules were paler than at the periphery. In some cells, the protoplasm was cloudy, the nuclei were irregular, and the fat content, in those in the middle zones of the lobules, had increased. Granular mitochondria appeared in cells of the central zone and in the vacuolated cells of the middle zone; in the periportal zone, however, the mitochondria were normal in that they were rodlike and evenly distributed throughout the cells.

At six hours, swelling of the cells of the liver was general in the central areas, and fatty degeneration and pyknotic nuclei were common (fig. 1). The endothelium of the central vein was swollen, often desquamated and frequently ruptured. In the portal areas, the cells were normal, whereas in the middle zone there was a ring of clear, vacuolated cells filled with fat, often without any visible protoplasm or

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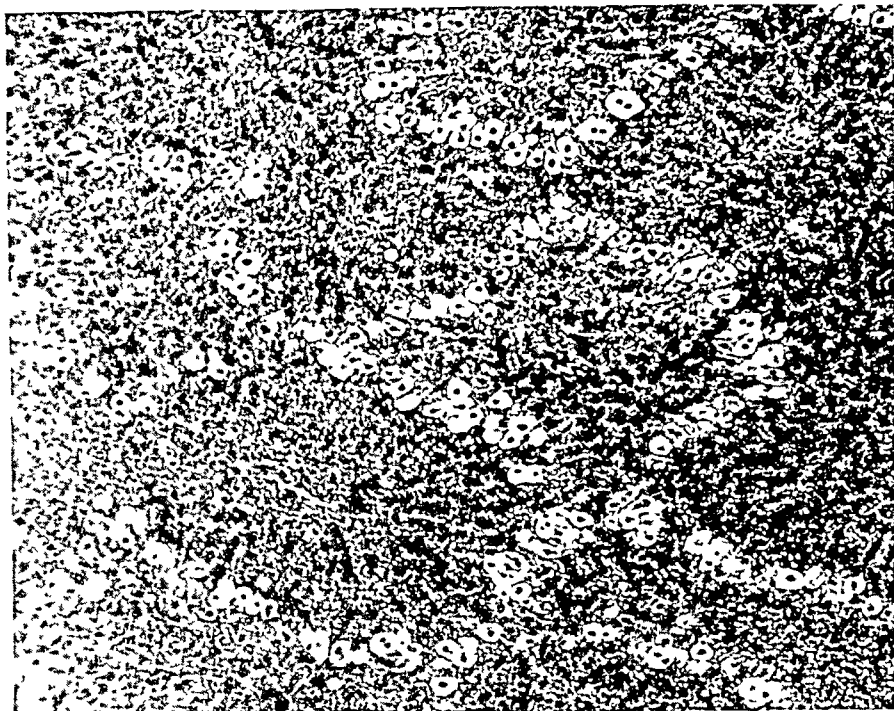


Fig. 1.—Liver of white rat six hours after oral administration of 0.25 cc. of carbon tetrachloride.

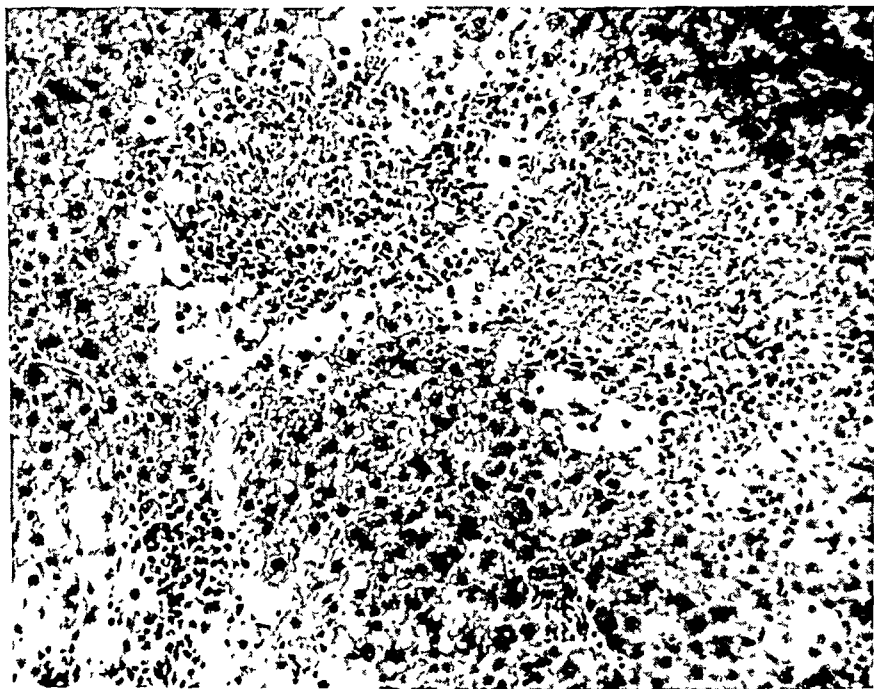


Fig. 2.—Liver of white rat twenty-four hours after oral administration of 0.25 cc. of carbon tetrachloride.

nuclei. Small droplets of fat occurred in the cells of the central zone, but they were practically absent from the cells at the periphery of the lobule. It was estimated that from 25 to 50 per cent of the parenchyma of the liver was loaded with fat. The mitochondria had disappeared from the large fat-containing cells of the middle zone, and from several cells in the central zone. In other cells of this zone, the mitochondria were degenerating, coagulated into clumps or thick granules around the nucleus (perinuclear condensation); in the remaining part of the cell, the protoplasm was coagulated and grossly granular. At the periphery of the lobule, however, the mitochondria remained rodlike and evenly distributed throughout the hepatic cells.

The maximal injury to the liver apparently was reached twenty-four hours after the administration of a single injection of the drug (fig. 2). All cells of the lobule were distended with either small or large clear fat vacuoles, and necrosis was marked in the central zones. Central veins were destroyed, and erythrocytes were widely distributed throughout the sinusoids. Lymphocytes, polymorphonuclear cells, mononuclear phagocytic cells, monocytes with horseshoe-shaped nuclei and many eosinophilic leukocytes had invaded the necrotic area. Kupffer cells had increased in number and had migrated toward the center of the lobule. Globules of fat, very irregular and often confluent, filled the central and middle zones, while at the periphery the fat droplets were more discrete and more uniform in size, and were within the cytoplasm of the hepatic cells. In some sections the veins were largely distended with fat. The mitochondria at twenty-four hours were normal in the periportal zone, but appeared as short, thick rods elsewhere, assuming a periglobular distribution (fig. 3). This arrangement of the mitochondria is probably due to a mechanical effect induced by the vacuolation of the protoplasm, for the mitochondria were pushed around the nucleus and against the cell membrane by the fat vacuoles. Nearer the central veins, the mitochondria were more infrequent, and they were entirely absent in the central zones, where there were only reticulum, fragmented nuclei and various infiltrating cells. Mitotic figures were identified at this stage in the periportal zone or even among the fatty cells, and indicated that an attempt at regeneration was in progress.

Two days after administration, the injury to the liver was still marked, although there was less fat at the periphery of the lobule, and the cells bordering on the portal spaces were again more nearly normal (fig. 4). The central vein had disappeared, and granular debris of coagulated protoplasm and hyaline degeneration represented all that remained of the central zones of the lobules.

After four days, the necrosis was restricted to a small central area, sharply demarcated from the remainder of the lobule. The fat content was reduced and restricted to the middle zone, and active phagocytosis

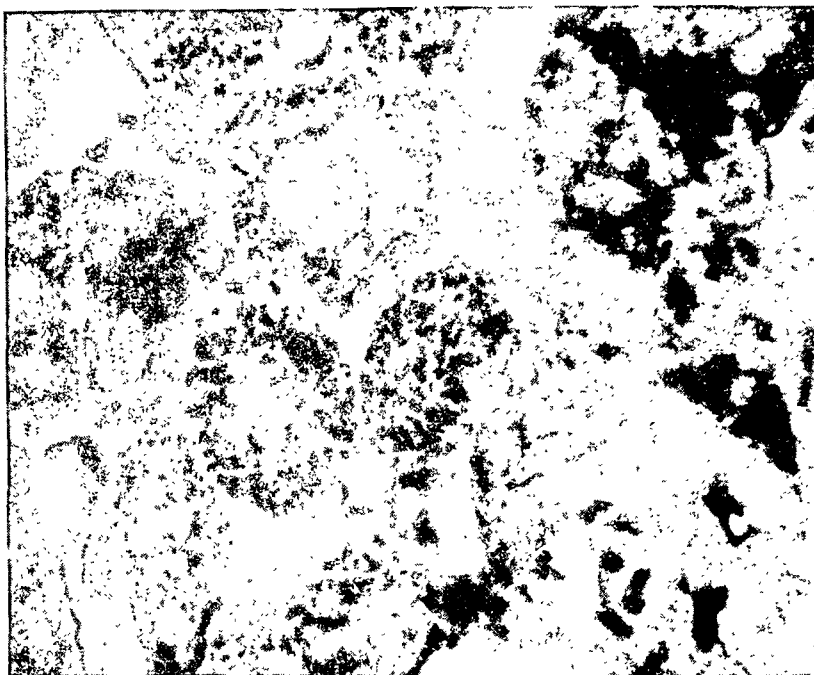


Fig. 3.—Concentration of mitochondria in hepatic cell of liver of white rat twenty-four hours after oral administration of 0.25 cc. of carbon tetrachloride.

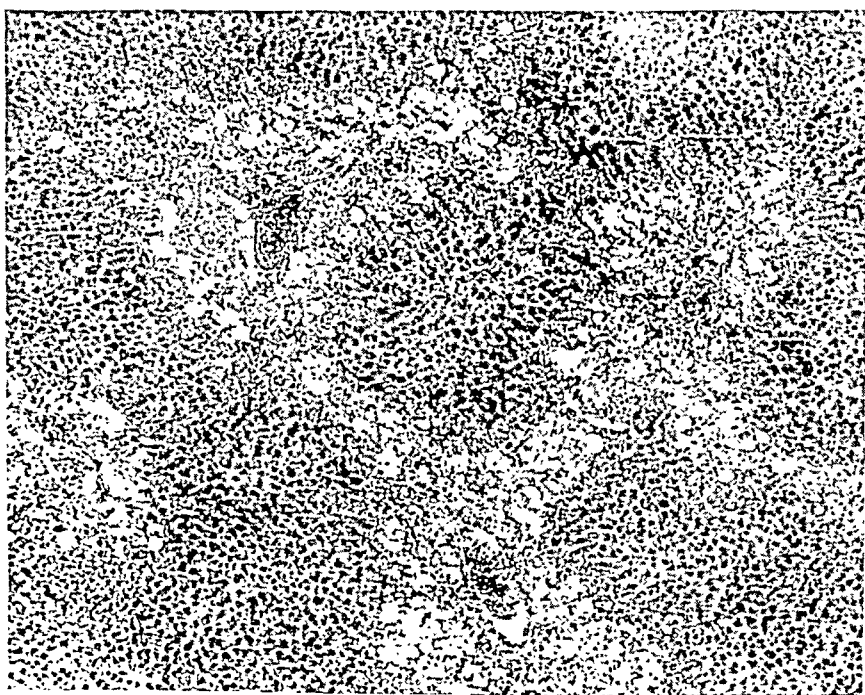


Fig. 4.—Liver of white rat two days after oral administration of 0.25 cc. of carbon tetrachloride.

had eliminated most of the cellular débris. There was considerable activity of the Kupffer cells, and the cells of the mesenchyme were still preponderant in the central zone. The mitochondria were again normal after four days, and after one week a few necrotic cells with some infiltrating lymphocytes remained in the center of the lobules. The walls of the central veins were incomplete in most instances, although their restoration from the uninjured portion of the endothelium was in progress. Necrosis was often sufficiently extensive completely to destroy the entire vein, so that a new endothelium developed from the cells of the mesenchyme which filled the central zones. The development of a definitely organized vascular channel was preceded by clefts or spaces within these infiltrating cell masses, and subsequently certain of these cells gave rise to attenuated protoplasmic processes which formed a clearly delineated wall, the endothelium of the new central vein. These cells of the mesenchyme were probably embryonic, and the organ may revert to embryonic principles in producing from undifferentiated mesenchyme a specialized and differentiated endothelium. Under normal conditions, these mesenchymal cells are usually restricted to the portal spaces, but the necrotic zone around the central veins induced mobilization in order to phagocytose the débris and to assist in the reconstruction.

It was clear from this preliminary study that injury to the hepatic lobule was microscopically evident in three hours following a single oral administration of 0.25 cc. of the drug. Destruction spread from the central portion toward the periphery and reached a maximum at twenty-four hours, when practically the entire lobule was involved.

With larger doses of 0.50, 0.75 or 1 cc. of carbon tetrachloride the injury to the lobule was far more extensive. Instead of a central area of coagulation and hyaline degeneration, there was a uniform diffuse lesion with cloudy swelling, pyknosis and granular and fatty degeneration over the entire lobule. Following a single injection of 0.75 cc., normal hepatic tissue was not identified after two days. Repair following larger doses of the drug took place more slowly, and the degree of injury was not proportional to the amount injected. A very large dose, such as 2 cc., killed the animals by a functional disturbance without extensive anatomic lesions in the liver. In the same way, the addition of alcohol to the drug increased its toxicity without inducing extensive pathologic lesions. The gross appearance of the liver rather closely conformed to the histologic detail. When the liver was injured by carbon tetrachloride, the organ was swollen, soft and friable. Central necrosis was indicated, grossly, by a delicate yellow tracing with distinct markings over the surface. The liver was yellowish brown or frequently, in cases of extreme injury, entirely yellow. Regeneration, however, induced a normal macroscopic appearance. As far as either

the macroscopic or the histologic appearance was concerned, there was no difference between the right and the left lobes of the liver.

In another series of rats, injections of 0.25 cc. of carbon tetrachloride were given twice a week for varying periods. From three to six injections produced lesions of the same sort as a single injection. Areas of hyaline degeneration and necrosis were invaded by erythrocytes and by excessive mononuclear infiltration, and they were surrounded by cells that were often distended by a single large fat vacuole and on the periphery by cells containing smaller vacuoles. The endothelium of the central veins was partially or entirely destroyed, and the central vein often appeared as a space surrounded by a thick layer of these cells of infiltration. Occasionally both the central and the portal areas became contiguous, and the necrosis, which was actually central in origin, resembled a periportal lesion.

The pathologic condition of the organ was not always proportional to the number of injections given; neither was it indicative of the prognosis for the life of the animal. The liver of a rat that received three injections and was killed four days later might be more severely injured than that of a rat receiving six injections that was killed two days later. In the same way, a rat that died spontaneously might have a better liver histologically than a rat killed while in good general condition at a longer interval after the injection. There was a marked increase in the number of bile ducts following repeated injections, often more apparent than real. Many groups of epithelial cells, often without lumen, as well as buds of cells indicated a real proliferation of new bile ducts. In cases of slight reaction, proliferating bile ducts were restricted to the portal spaces, but whenever an area of central necrosis reached a portal space, the bile ducts proliferated into this extensive necrotic zone.

The demonstrable fat content of these injured livers was very high. Fat globules were found even in the cells undergoing mitosis, and cells around the portal spaces were speckled with small globules of fat. The numbers of fibroblasts in the zones of infiltration suggested early development of connective tissue with fibrosis. With special staining technic, layers of connective tissue were identified around the central vein, and fibers followed the axis of the bands of infiltration into the necrotic zones.

After from ten to thirty injections, however, the connective tissue reactions had increased (fig. 5). There was definite fibrosis, which disturbed the usual lobulation and divided the parenchyma into small separate and distinct islands of pseudolobules. These islands of hepatic cells, largely vacuolated with fat, were encircled by fibrous tracts.

Repeated poisoning appeared to exhaust and destroy the regenerating power of the hepatic parenchyma and at the same time stimulated

the mesenchyme to more active proliferation. Regeneration and cirrhosis were not secondary to each other, but represented two separate reactions produced by the toxic agent. The capacity for regeneration and the extent of cirrhosis following repeated injections were inversely proportional to each other. Whereas regenerative capacity decreased, so that the liver lost its replacement power, the fibrous reaction rapidly increased and produced marked cirrhosis.

Following repeated injections, livers were macroscopically large, with yellow tracings throughout. They were no longer soft, but exceedingly firm and hard, and presented a hobnail roughness on the surface.

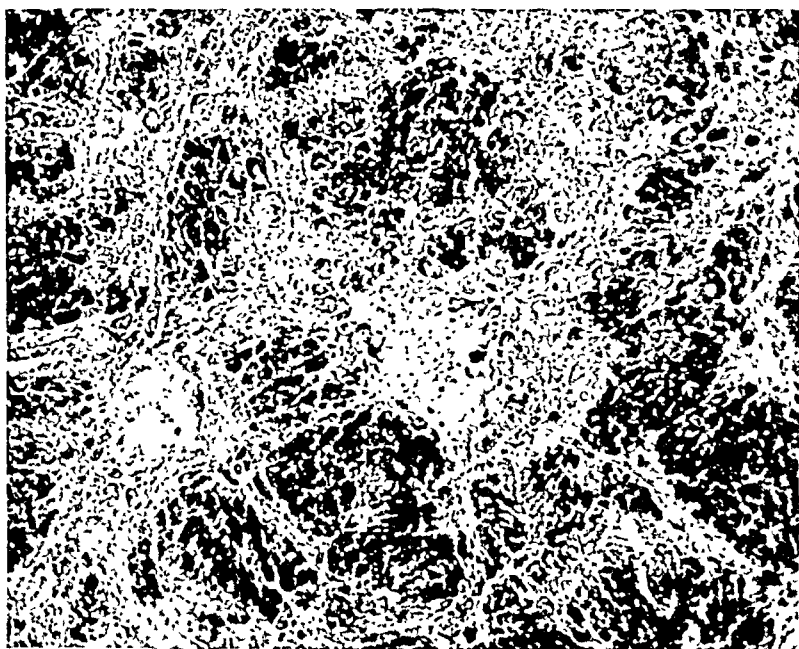


Fig. 5.—Liver of white rat after oral administration of eleven doses of 0.25 cc. of carbon tetrochloride (two doses a week).

The macroscopic and histologic lesions at this stage bore the first evidence of the typical atrophic cirrhosis with circulatory disturbance which the frequent repetition of injury from carbon tetrachloride brings about in the liver.

*Effects of a Single Dose of Carbon Tetrachloride Before Partial Hepatectomy.*—In order to study the combined effect of chemical injury and partial removal, 0.25 cc. of carbon tetrachloride was given to each of a large number of rats. Twenty-four hours later, when the lesion was extensive and the regenerative process had begun, two lobes of the liver were removed. At intervals after the operation, three rats were killed, and the remnants of liver were removed for study.



Twenty-four hours after the operation, or two days after the carbon tetrachloride was given, the lesions of central necrosis were as extensive as those occurring twenty-four hours after a single dose of carbon tetrachloride had been given without partial hepatectomy. The types of injury to the cytoplasm, the nuclei and the mitochondria were essentially like those described. The lesions were not definitely restricted to the center of the lobule, and degenerating cells with pyknotic nuclei occurred throughout the lobule.

Two days after the operation, or three days after the carbon tetrachloride was given, the injury was comparable to that seen two days after the administration of the drug alone. It would seem that the detoxifying factor which may exist in the normal liver was reduced in the animals operated on, for the organ was less resistant and the process of recovery retarded. This retardation, however, was only manifest during the first two or three days, for on the fourth day after the operation the liver had overcome this first inhibition and was in active regeneration. At one week, repair of the liver was as marked in partially hepatectomized animals as in those clinically injured. It is of interest that in the livers in a state of restoration, numbers of fibroblasts among the infiltrating cells had given rise to connective tissue fibers around the central veins, so that after this first brief period of delay restoration progressed with its sequence of hypertrophic nuclei, mitosis and nests of hematopoietic cells, budding bile ducts and marked mesenchymal and reticulo-endothelial activity. It seems that the combined action of restoration and regeneration had induced more intense activity of the mesenchyme and especially of the Kupffer cells.

*Effects of a Single Dose of Carbon Tetrachloride After Partial Hepatectomy.*—In order to study the effects of carbon tetrachloride on the liver during restoration, we gave a single injection of the drug to several groups of animals at varying intervals after the surgical removal of about 65 per cent of the liver.

The first group included animals operated on that received the drug before the hepatic remnant had regained its normal weight and volume (that is, before two weeks after partial removal). It is difficult to draw any definite conclusion from these experiments concerning the susceptibility of the organ to the drug, since the substance of the liver was only a third or a half of the original normal amount. The injection was in a measure equivalent to the administration of a double or a triple dose.

The animals of the second group received carbon tetrachloride after the remnant of liver had attained its normal volume, but before restoration was complete histologically (that is, between the second and fourth weeks after partial removal). In these animals the liver was a new, rapidly growing parenchyma with high metabolic activity, and it

possessed at least some embryonic qualities. Rather striking results were obtained in these experiments.

Twenty-four hours after 0.25 cc. of carbon tetrachloride was given to these animals, central necrosis and fatty degeneration were far less extensive than in the normal liver that had been subjected to injury (fig. 6). The nuclei were pyknotic, and there was slight round cell infiltration in the region. The central veins were incomplete or defective, and hemorrhage had occurred. Fat globules filled the cells of the middle zone, but the periportal zones were free, and the portal spaces were intact. This injury of twenty-four hours in the restored liver was similar to a six hour lesion in the normal liver. The hepatic cells were

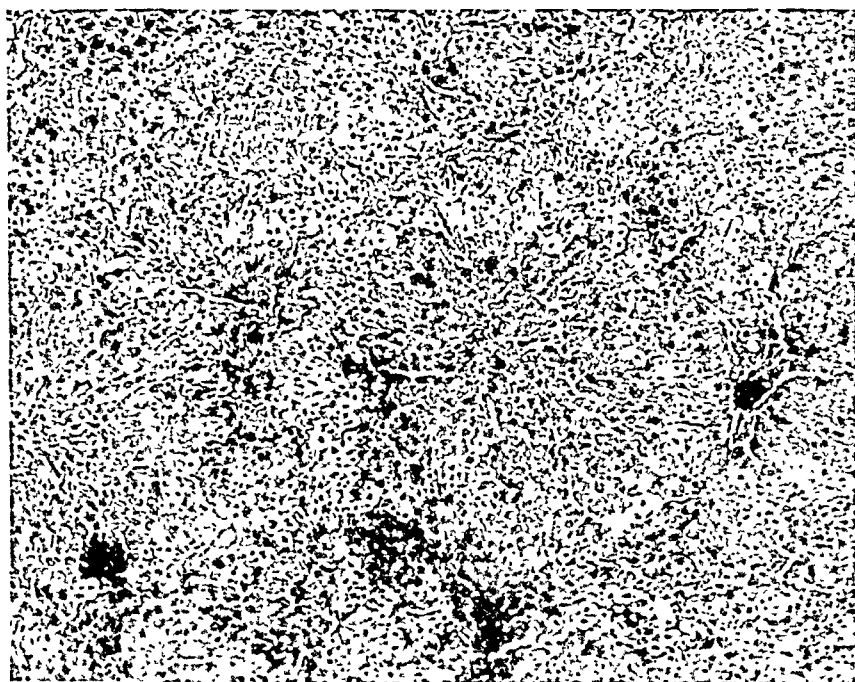


Fig. 6.—Liver of white rat, four weeks after partial hepatectomy and twenty-four hours after oral administration of 0.25 cc. of carbon tetrachloride.

large, and a fair number of mitotic figures were visible all over the lobules. Normal rodlike and granular mitochondria occurred in the cells at the periphery and in the middle zone, and as a whole the lesion was less extensive, and regeneration and repair were completed within a short period.

Unquestionably, there was a significant difference in the effect of the toxic agent on the normal and on the newly restored liver. The latter was more resistant to the drug and seemed to have a higher detoxifying ability. Several weeks after partial hepatectomy, the liver might again be considered normal, in that the effects of carbon tetrachloride were essentially identical with those of the normal liver.

## COMMENT

It is quite clear that the cirrhosis produced by carbon tetrachloride cannot be caused by necrosis, for the production of connective tissue, after a certain number of doses of carbon tetrachloride, is not dependent on the extensive necrosis. Fibrous tissue was produced with very small repeated injections, which caused little necrosis, but it did not appear in a liver that had been almost destroyed by one massive dose. A slight connective tissue reaction occurred following a single injection of carbon tetrachloride, but it increased as the stimulation was repeated more frequently. Van Heukelom,<sup>7</sup> in 1896, demonstrated that necrosis and cirrhosis are independent of each other, although they may be induced by the same pathogenic factor. Cirrhosis, in the later stages, can impair the vitality of hepatic cells by compression, so as to cause necrosis.

The early localization of fat in the hepatic lobule after the administration of carbon tetrachloride may be contrasted with the observations of Fiessinger<sup>8</sup> on periportal lesions induced by chloroform. The areas of vacuolated cells appearing six hours after the administration of carbon tetrachloride and persisting for at least twenty-four hours were not observed after the administration of chloroform,<sup>9</sup> phosphorus<sup>10</sup> or any other toxic agent.<sup>11</sup> With a few exceptions, all authors describe injury from chloroform as central,<sup>12</sup> and injury from phosphorus as periportal. As was suggested by Loeffler,<sup>13</sup> a poorer blood supply may serve to explain the localization of the fat in the middle zone of the lobule. However, I did not observe vasoconstriction, and the stasis of the blood described by Loeffler as central after chloroform poisoning was more uniform over the entire lobule following carbon tetrachloride poisoning. Finally, both the central necrosis and the central origin of the cirrhosis after the administration of carbon tetrachloride rather nullify the opinions of Noël and Rosier,<sup>14</sup> who explained the periportal action of any toxic agent by the histophysiology of three zones of the lobule.

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12. Loeffler, L., and Nordmann, M.: *Virchows Arch. f. path. Anat.* **257**:119, 1925.

13. Loeffler, L.: *Virchows Arch. f. path. Anat.* **265**:41, 1927.

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In cases of severe injury I found large amounts of fat in the hepatic veins and in the bile ducts coincident with the postmortem observations of MacMahon and Weiss.<sup>15</sup> In a case of carbon tetrachloride poisoning, these authors described the presence of many fat globules in the central and middle zones of the lobules of the liver, as well as in the hepatic veins. Fat even occurred in the blood of the right side of the heart, and the larger pulmonary arteries contained as much as 60 per cent fat.

The reactions of mitochondria to carbon tetrachloride correspond to the description of Mayer, Rathery and Schaeffer<sup>16</sup> and Okushi;<sup>17</sup> they described protoplasmic cytolysis and chondriolysis and homogenization. Mitochondria are most labile and show the earliest effects of the injury, but before they disappear completely, they prove more resistant than their initial action would indicate.

The proliferation of bile ducts and new parenchyma after partial hepatectomy and chemical destruction of the liver, together with the appearance of infiltrating cells, indicates a return to an embryonic condition. Budding and branching bile ducts produce a tubular type of gland, and these cells may gradually transform into parenchymal cells. Many authors admit the participation of the bile ducts in the regeneration of hepatic cells. This procedure, however, plays a secondary part, for mitotic figures in the parenchyma account for the production of many new cells, whereas, contrary to Ponfick<sup>18</sup> and others, mitotic figures were not observed in the epithelial cells of the budding bile ducts. But under conditions of severe injury, such as several doses of carbon tetrachloride may induce, mitosis was never seen, and it may be that hepatic parenchyma is maintained by the actively proliferating biliary epithelium.

#### SUMMARY AND CONCLUSIONS

The purpose of this investigation was to determine whether the properties of the restored liver after partial removal were different from those of the normal organ. The effect of carbon tetrachloride on the restored liver was compared with its effect on the normal organ. It was found that when a single administration of 0.25 cc. of carbon tetrachloride was given by stomach tube to a normal rat, weighing from 150 to 200 Gm., central necrosis and fatty degeneration of the liver ensued. The maximal lesion was reached after twenty-four hours, and the liver was completely regenerated after two weeks. Repeated injections, however, induced typical cirrhosis.

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16. Mayer, A.; Rathery, F., and Schaeffer, G.: *J. de physiol. et de path. gén.* **16**:581, 1914.

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From the standpoint of the effects of carbon tetrachloride following partial removal of the liver three conclusions were apparent: 1. When chemical injury preceded partial hepatectomy, recovery was greatly retarded. 2. When chemical injury was induced from two to four weeks following partial removal, the lesion was far less marked and recovery ensued far more rapidly than in the normal liver following administration of the drug; the restored liver appeared to be considerably resistant to the toxic influence of the drug. 3. When chemical injury was induced two months after partial removal, the extent of the lesion was more or less identical with that in a normal liver following administration of carbon tetrachloride.

# MORPHOLOGY OF THE INFLAMMATORY DEFENSE REACTIONS IN LEUKEMIA

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Since the cells of an exudate are chiefly derived from the circulating blood, it is to be expected that profound changes in the cellular composition of the blood will manifest themselves in the morphology of the inflammatory defense reactions. Thus, in spontaneous or experimental agranulocytic conditions, inflammation is characterized by the severity and the predominance of the alterative process and the lack of cellular response, and though histiocytes and lymphocytes occasionally accumulate about the necrotic areas, they are unable to compensate for the loss of the granulocytes (Rotter,<sup>1</sup> Mönckeberg,<sup>2</sup> Silberberg,<sup>3</sup> Winternitz and Hirschfelder<sup>3a</sup> and others). If only a few granulocytes are left, they are quickly drawn to the focus of inflammation and help in localizing it (Silberberg).

Histologic studies on inflammation in leukemia are few, and the observations recorded are contradictory. The finding most frequently noted is that of the predominance of polymorphonuclear leukocytes in the exudate of cantharidin blisters in cases of lymphatic leukemia (Litten,<sup>4</sup> Neumann,<sup>5</sup> Sonnenfeld and Leffkowitz<sup>6</sup> and others). Askanazy<sup>7</sup> and Naegeli<sup>8</sup> described neutrophilic leukocytes in the gangrenous lesions of the oral cavity in acute leukemia. Rodler-Zipkin<sup>9</sup> studied a case of acute large-celled lymphatic leukemia in which there was a suppurating sinus in the region of the left knee, and found in the granulation tissue lining this sinus many leukocytes. According to Naegeli, the appearance of granulocytes in inflamed areas in cases of

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1. Rotter, W.: *Virchows Arch. f. path. Anat.* **258**:17, 1925.

2. Mönckeberg, J. G.: *Verhandl. d. deutsch. path. Gesellsch.* **16**:150, 1913.

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3a. Winternitz, M. C., and Hirschfelder, I. B.: *J. Exper. Med.* **17**:657, 1913.

4. Litten, cited by Dionisi.<sup>11</sup>

5. Neumann, cited by Dionisi.<sup>11</sup>

6. Sonnenfeld, A., and Leffkowitz, A.: *Klin. Wchnschr.* **9**:548, 1930.

7. Askanazy, M.: *Virchows Arch. f. path. Anat.* **137**:1, 1894.

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lymphatic leukemia depends on the presence of remnants of granulopoietic tissue in the bone marrow. If the bone marrow is completely devoid of granulocytes, not a single pus cell can be detected in the foci of inflammation. This was the case in an acute lymphoblastic leukemia reported by Schultze<sup>10</sup> in which there were shown numerous hemorrhagic blisters of the skin. The blisters contained many large mononuclear cells and streptococci, but pus cells were absent. A similar substitution of neutrophilic leukocytes by leukemic cells in acute inflammation was observed by Dionisi,<sup>11</sup> who concluded from his findings that the characteristic elements of lymphemic blood were able to migrate through the wall of the blood vessels if the leukemic organism was confronted with an inflammation. Bickhardt,<sup>12</sup> too, stated that the leukemic patient reacts to an inflammatory irritation in the same manner as a normal person, the pathologic blood cells, however, substituting the polymorphonuclear leukocytes in the exudate.

In discussing the relations between inflammation and leukemia, one has to take into consideration the fact that an intercurrent infection often influences the blood picture and also the cellular composition of the blood-forming organs, establishing more or less normal conditions (Dock,<sup>13</sup> Hirschfeld,<sup>14</sup> Naegeli<sup>8</sup> and others). The leukemic organism does not seem to have lost the ability to produce normal blood cells (Hirschfeld), and this ability can be awakened by bacterial toxins (H. F. Müller<sup>15</sup>).

Little attention has so far been given to the productive inflammatory processes in leukemia save the combination between leukemia and tuberculosis, which will be discussed in a later publication. Since the morphology of leukemia is characterized by a profound change in the blood-forming potencies of the mesenchyma, it will be of special interest to investigate how this abnormal mesenchyma reacts to an inflammatory stimulus. Does it reveal these abnormal potencies when irritated, or does its reaction not differ from that of normal mesenchyma?

During the last three years I have collected among thirty cases of leukemia ten in which, at autopsy, inflammatory lesions were found, and in which the autopsy was done so shortly after death as to secure perfect fixation. The histologic descriptions are based mainly on

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10. Schultze, W.: Beitr. z. path. Anat. u. z. allg. Path. **39**:252, 1906.

11. Dionisi, A.: Folia haemat. **7**:368, 1909.

12. Bickhardt, K.: Folia haemat. **32**:83, 1926.

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14. Hirschfeld, H.: Ueber die Komplikation der chronischen Leukaemie mit anderen Krankheiten, in Schittenhelm, A.: Handbuch der Krankheiten des Blutes und der blutbildenden Organe, Berlin, Julius Springer, 1925, vol. 1, p. 334.

15. Müller, H. F., cited by Hirschfeld.<sup>14</sup>

material fixed after Helly and Maximow and stained after Giemsa and fixed with a solution of formaldehyde U. S. P. (1:10) and stained for stable oxydase.

#### LYMPHATIC LEUKEMIA

*CASE 1.—Confluent Bronchopneumonia in a Case of Subacute Lymphatic Leukemia.*—A white boy, aged 6, had been suffering from profuse night sweats and a frequent, nonproductive cough for two years. Seven weeks before admission to the hospital, illness developed following the extraction of a tooth. The mother noticed that the child's abdomen became larger, and that he looked pale. Numerous bluish, pinpoint-sized to nickle-sized spots appeared all over the body and faded in two weeks. There was a persistent discharge from the nose with the formation of thick crusts.

On admission, the patient's temperature was 101.6 F., the pulse rate was 100, and the respiratory rate was 24. The lymph nodes on both sides of the neck, especially on the right side, and in both axillae and groins were bean-sized, discrete and soft. The breath sounds throughout the lungs were exaggerated, and a rumbling systolic murmur was heard over the pulmonary area. The lower edge of the liver could be felt two fingerbreadths below the costal arch. The spleen was not palpable.

The erythrocyte count was 1,260,000; the hemoglobin content (Newcomer) was 22.5 per cent; the white blood cell count was 9,725, with lymphoblasts 74.5, lymphocytes 24.5 and neutrophils 1 per cent. The platelet count was 62,000. Marked anisocytosis and poikilocytosis were present; there were 6 normoblasts per two hundred white cells.

The child remained in the hospital for six weeks, during which the white count fluctuated between 3,200 and 31,000.

*Autopsy:* There was slight hyperplasia of the cervical, axillary, inguinal, peripanicreatic, peribiliary, periaortic and mesenteric lymph nodes; the largest of the nodes measured 15 by 15 by 10 mm. The spleen weighed 155 Gm. and was moderately firm; the pulp was bright purple-red with small follicles. The liver weighed 1,070 Gm.; the heart, 145 Gm. Severe generalized anemia was present. Extensive hemorrhages were found about and in both kidneys; the kidneys weighed 590 Gm. Other changes were: severe fatty changes of the myocardium and liver; petechial hemorrhages in the skin, pericardium, endocardium, renal pelvis, suprarenal cortex and urinary bladder; hydropericardium; hydrothorax of the left side; focal bronchopneumonia of the left lower pulmonary lobe, and confluent bronchopneumonia of the right lower lobe.

*Histologic Observations:* Microscopic examination of the organs showed the typical picture of large-celled lymphatic leukemia with dense infiltrations in the splenic pulp, in the periportal septums of the liver and in the stroma of the kidneys and pancreas. There were smaller infiltrations in the lung and underneath the epicardium and endocardium. The lymph nodes were almost exclusively composed of the large cells, and there were only a few remnants of the secondary follicles. In the spleen, the malpighian bodies could be distinguished from the pulp, since they were formed by small lymphocytes. The bone marrow was a uniform accumulation of large lymphoid cells. The type cell of all the infiltrations was a cell about from 12 to 14 microns in diameter. The nuclei were round or slightly indented, contained evenly distributed small chromatin granules and were surrounded by scanty light blue cytoplasm, which was free from oxydase granules. These cells were mixed with a varying, but usually small number of small lymphocytes and plasma cells. In the bone marrow, in the splenic pulp and especially in



the renal infiltrations there were many foci of erythropoiesis composed of erythroblasts and large orthochromatic normoblasts. The bone marrow and the splenic pulp also contained small groups of neutrophilic myelocytes and mature leukocytes. In the bone marrow and in the renal infiltrations, single well preserved megakaryocytes were present.

In striking contrast to the scarcity of the granulocytes in the other organs, the alveolar exudate in the pneumonic areas was composed chiefly of mature polymorphonuclear leukocytes, which were embedded with a fine net of fibrin and were mixed with a few macrophages and erythrocytes (fig. 1). The lumen of the small

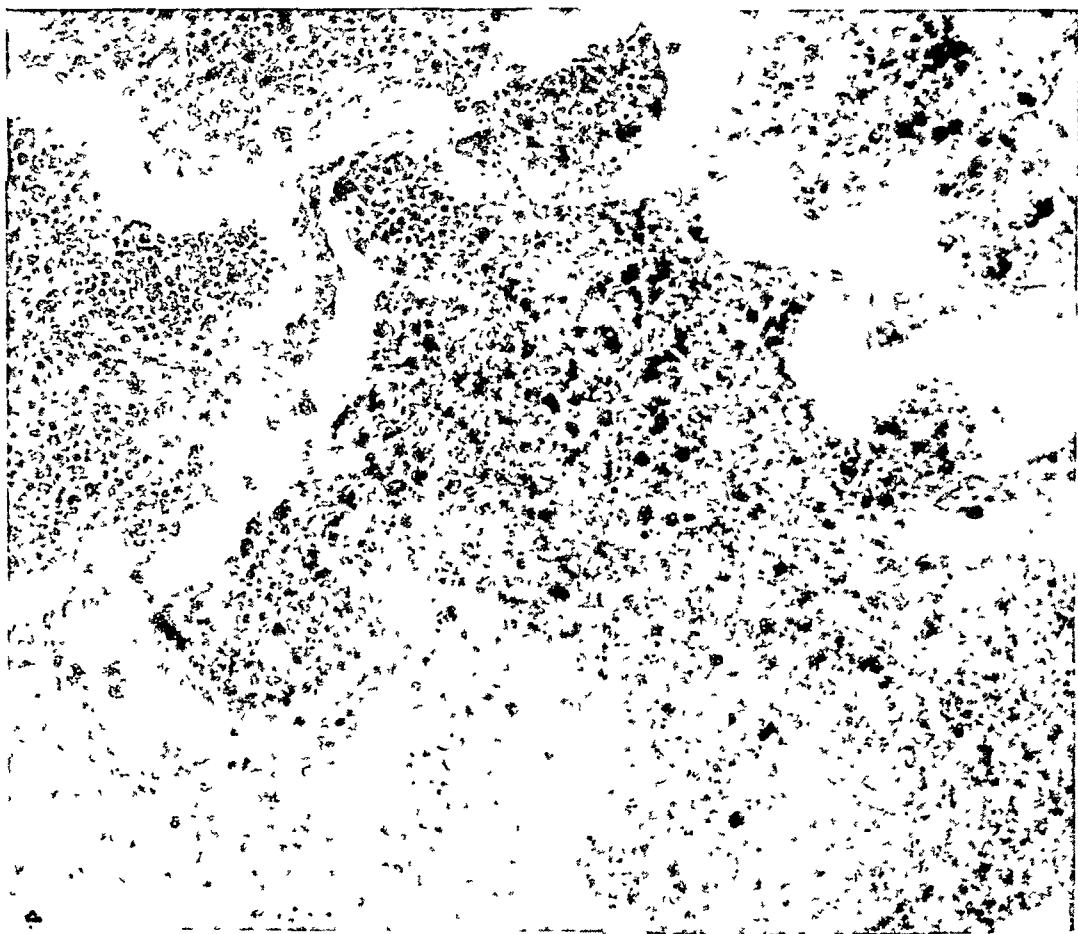


Fig. 1 (case 1).—Pneumonic area. The exudate is chiefly composed of granulocytes; oxydase reaction;  $\times 150$ .

bronchi and bronchioli was occluded by plugs of fibrin. The alveolar capillaries contained single granulocytes and a moderate number of lymphoid cells.

*CASE 2.—Serofibrinous Peritonitis Following a Splenic Infarct in a Case of Chronic Lymphatic Leukemia.*—A white man, aged 57, was admitted to the hospital stating that he had lost 50 pounds (22.7 Kg.) in weight during the last eight months. He also had noted an enlargement of the lymph nodes and complained of severe pains in the arches of his feet. Shortly prior to his admission he had a severe attack of sore throat. On admission, the temperature was 98 F.; the pulse rate was 120, and the respiratory rate, 24. The blood pressure was 158 systolic and 87 diastolic. The anterior and posterior cervical lymph nodes and

the submandibular, inguinal and axillary nodes were pea-sized, soft, discrete and painless. The lower pole of the spleen could be palpated 5 fingerbreadths below the costal arch, and the lower border of the liver was felt  $2\frac{1}{2}$  fingerbreadths below the costal arch.

The erythrocyte count was 3,250,000; the hemoglobin content (Dare) was 60 per cent. The white blood cell count was 139,000, with lymphocytes (about one and one-half the size of normal lymphocytes with coarsely trabeculated nuclei and narrow rims of deeply basophilic cytoplasm) 98 per cent and lymphoblasts (three times the size of lymphocytes) 2 per cent. Under treatment with benzene, the lymph nodes and the spleen became smaller, and the white count dropped to 98,000. The lymphocytes diminished to 95.5 per cent, and 2.5 per cent neutrophils were counted.

In the fifth week of the patient's stay in the hospital, he suddenly began to have a severe pain in the region of the spleen, and the temperature rose to 102 F. Two days prior to this attack he had a sore throat. He became rapidly weaker and died four days after the onset of the abdominal pain.

Autopsy: Post mortem, diffuse serofibrinous peritonitis with 400 cc. of free exudate was found. The exudate contained many typical pus cells and very few lymphoid cells, and on being cultured yielded a hemolytic streptococcus. The spleen weighed 1,810 Gm. and was covered by a thick, soft, fibrinous membrane; in the lower third of the posterior margin there was a recent light purple-gray, sharply demarcated anemic infarct. The liver measured 30 by 20 by 10 cm., was soft and pale brown, and contained single pinhead-sized whitish nodules. All the lymph nodes were enlarged, and the largest nodes, which measured 40 by 35 by 15 mm., were found along the extrahepatic bile ducts, the aorta and the iliac vessels. The bone marrow was moderately firm and purplish pink mottled with darker purple-red. Cholelithiasis was present. There was septic staining of the intima of the aorta.

Histologic Observations: Microscopically, the bone marrow was made up chiefly of oxydase-negative lymphoid cells, the majority of which were slightly larger than a lymphocyte. The nuclei contained coarse chromatin granules. There were single large, deeply basophilic round cells and a few plasma cells. Around the blood vessels, numerous nests of young neutrophilic and oxyphilic myelocytes were found. The origin of these cells could be traced to the reticular adventitial cells. There were no relations between the granulated elements and the lymphoid cells. In addition to the granulopoietic areas, there were small foci of erythropoiesis and many young and mature megakaryocytes. The spleen showed an enormous predominance of the lymphoid cells and very few other elements, namely, free histiocytes, neutrophilic and oxyphilic myelocytes and an occasional plasma cell. The infarcted area, however, was surrounded by a zone of oxydase-positive cells with bean-shaped and lobulated nuclei. The larger veins leading to the infarct were occluded by thrombi composed of fibrin and lymphoid cells. Gram stain revealed in the infarct numerous streptococci.

The structure of the lymph nodes was obscured by a diffuse accumulation of the lymphoid cells described in the bone marrow. In the peribiliary, peripancreatic and splenic hilus lymph nodes there were many neutrophilic myelocytes and leukocytes and a few oxyphilic granulocytes. They were present in the distended sinuses as well as between the lymphoid cells. The sinuses also contained many streptococci. In the liver, the periportal tissue and the walls of the sublobular veins were densely infiltrated by the lymphoid cells, which also filled the portal capillaries. In the periphery of the periportal infiltrations and in some of the portal capillaries, large round cells were found, the cytoplasm of which contained

fine, purple-pink granulation. A few of these cells had coarse, oxyphilic granulation. Where the granulated elements filled the portal capillaries, the adjacent Kupffer cells contained similar granulation. The kidneys showed focal perivascular accumulations of lymphoid cells, and these cells also filled the tufts of the glomeruli. The lymphatic tissue of the pharynx and intestine was unchanged.

CASE 3.—*Streptococcic Septicemia Complicating Subacute Lymphatic Leukemia*.—A Filipino, 19 years of age, on admission to the hospital on Nov. 26, 1929, reported that he had had his tonsils removed one month before, and that a week later his neck increased in size, first on the right side and then on the left side, and that this swelling caused him difficulties in swallowing and breathing. The temperature was 98.6 F.; the pulse rate was 60, and the respiratory rate, 20. External examination revealed a generalized lymphadenopathy, most marked in the neck. The glands ranged in size up to that of a walnut, and were matted together but not adherent to the skin. The result of the Kahn test was negative. The x-ray picture of the chest was negative.

The erythrocyte count was 3,800,000; the hemoglobin content (Dare) was 55 per cent. The white blood cell count was 10,000, with neutrophils 70, lymphocytes 25 and monocytes 5 per cent.

Biopsy of a cervical lymph node showed lymphatic leukemia. The clinical diagnosis was: aleukemic lymphadenosis. Under treatment with the x-rays, the patient's condition improved markedly, and he returned home on Dec. 21, 1929. He was readmitted to the hospital on Feb. 17, 1930, acutely ill, feeling very weak with dull aching pains over the entire body. He felt hot and had nosebleed about once a day, losing a teaspoonful of blood. He complained further of severe frontal headache, accompanied by dizziness and spots before the eyes, and a constant cough productive of dark red blood. His temperature was 103.4 F.; his pulse rate was 110.

The lymph nodes were distinctly larger than on his first admission. There were petechial spots over both eyelids and on the hard palate. The gums were swollen and bluish, and there was marked mediastinal dulness. A systolic murmur with a systolic thrill was heard over the entire precordium. The spleen and liver were not palpable.

The erythrocyte count was 1,500,000; the hemoglobin content, 40 per cent. The white blood cell count was 53,000, with lymphoblasts 79, lymphocytes 16 and neutrophils 16 per cent. There were slight anisocytosis and poikilocytosis, with 2 nucleated red cells per hundred white cells. While the patient was in the ward, the nosebleeds became more and more severe, and the temperature remained around 103 F. One day before death, the erythrocyte count was 980,000; the hemoglobin content, 15 per cent. The white count was 850 (1), with lymphoblasts 78 and lymphocytes 22 per cent. On Feb. 25, 1930, the patient died.

Autopsy: At autopsy, the peripheral, thoracic and abdominal lymph nodes were uniformly enlarged, reaching a diameter of 50 by 20 by 25 mm. They were soft and spongy and varied in color from deep purple-gray to reddish gray. The bone marrow was semiliquid and purple-gray. The thymus weighed 26 Gm. and was light pinkish gray. The spleen weighed 170 Gm. It was very soft and dark purple-gray with small light gray follicles. The liver weighed 1,430 Gm. Other changes were: severe fatty degeneration of the myocardium and liver, anemia of the kidneys, gangrenous stomatitis and petechial hemorrhages underneath the epicardium and endocardium and in the gastric mucosa and renal pelvis.

Histologic Observations: Microscopically, the lymph nodes appeared very loose, and the normal structure was completely obscured. The sinuses and capillary blood vessels were much dilated, and the reticulum was very prominent

because of disintegration of the lymphatic cells. The majority of these cells, which were slightly larger than lymphocytes, had nuclei the chromatin of which had been separated into coarse granules and the membrane of which had disappeared. Single large round cells with pale nuclei and ample, basophilic cytoplasm had escaped the nuclear disintegration. The cytoplasm of these cells contained fine, oxydase-positive granulation. In the abdominal lymph nodes, the granulated cells were more numerous than in the other lymph nodes, and there were also small nests of erythroblasts and normoblasts and single megakaryocytes. In the spleen, the breaking up of the nuclei of the lymphoid cells was even more striking than in the lymph nodes, and in many places only the reticular cells of the cords and the lining cells of the sinuses were left. In the follicles, too, the lymphoid cells were severely affected. The periportal tissue of the liver contained numerous lymphoid cells, which were found also about the sublobular veins and in the portal capillaries. In the latter location, they formed small groups. The karyorrhexis of the nuclei was less marked than in the lymph nodes and spleen. About 94 per cent of the cells of the bone marrow were lymphocytes with narrow rims of cytoplasm and nuclei showing a coarse chromatin net. The majority of the cells were well preserved, and only a few larger lymphoid cells revealed the nuclear changes previously described. There were 3.8 per cent plasma cells, 3.8 per cent normoblasts and 0.8 per cent oxyphilic granulocytes. About the vessels at the corticomedullary border of the kidney, accumulations of lymphoid cells were found. These cells also dominated in the thymus, and Hassall's bodies were scanty. Gram stain demonstrated in the lymph nodes, spleen and bone marrow long chains of streptococci. Pieces taken from the swollen gums showed deep necrosis with an enormous number of cocci, fusiform bacilli and spirochetes. Underneath the necrosis there were extravasations of blood and a few loosely scattered lymphoid cells. No granulocytes could be detected. The picture was similar to that seen in agranulocytosis or panmyelophthisis. Cultures of the cardiac blood demonstrated hemolytic streptococci.

#### MYELOGENOUS LEUKEMIA

CASE 4.—*Suppurative Prostatitis in a Case of Acute Promyelocytic Leukemia.*—The clinical data of this case are incomplete. A white man, aged 34, complained of general malaise, headache and chilly feeling. The axillary and inguinal lymph nodes were cherry-sized. The lower pole of the spleen was at the costal arch. Petechial hemorrhages were found over the neck, shoulders and thighs. The duration of the illness was thirty days. The result of the Wassermann and Kahn tests was four plus.

The erythrocyte count was 1,020,000; the hemoglobin content (Dare), 32 per cent. The white blood cell count was 6,100, with myeloblasts 15, promyelocytes 31, neutrophilic myelocytes 6, neutrophils 18, eosinophilic myelocytes 3, eosinophilic leukocytes 1, lymphocytes 24 and monocytes 2 per cent. Marked anisocytosis and poikilocytosis were present, with 1 normoblast per hundred white cells. There were 15 per cent reticulated erythrocytes.

Autopsy: Post mortem there was shown moderate enlargement of the spleen (390 Gm.) and of the liver (2,065 Gm.), eccentric hypertrophy of the heart (410 Gm.) with small hemorrhages in the papillary muscles of the left ventricle, enlargement of the peripancreatic, peribiliary and axillary lymph nodes, marked generalized anemia and petechiae in the skin and epicardium. The prostate measured 6 by 3.25 by 2.5 cm. and was firm. In the left lobe there was a cavity 2 cm. in diameter, which was filled by thick, greenish pus. In the right lobe, a similar but smaller cavity was found.

**Histologic Observations:** The bone marrow showed a predominance of young neutrophilic myelocytes with many mitoses (44.8 per cent). There were many small groups of myeloblasts and oxyphilic myelocytes and very few nucleated red cells and megakaryocytes. There were 14.2 per cent lymphocytes and 4.5 per cent plasma cells. The abdominal lymph nodes revealed advanced myeloid metaplasia, chiefly neutrophilic promyelocytes. In the axillary and inguinal nodes, the myeloid transformation was less marked. The splenic pulp contained an enormous number of large round cells with round, light nuclei and fine, purple-pink granulation. There were a few myeloblasts, normoblasts and oxyphilic granulocytes. The follicles were small and lymphocytic. In the liver, the periportal tissue and the portal capillaries were filled by young, oxydase-positive cells of promyelocyte type. Small foci of granulopoiesis were present in the myocardium about the hemorrhages, in the stroma of the kidney and pancreas, in the medulla of the suprarenal gland, in the mucosa of the colon and about the blood vessels of the lung.

The stroma of the prostate was infiltrated by oxydase-positive cells with round, bean-shaped and lobulated nuclei. Similar cells were found in the lumen of some of the glands. There were areas of diffuse suppuration. The differential count of these areas was: mature neutrophilic leukocytes, 94.5 per cent, and myelocytes, 5.5 per cent. The differential count of the infiltrations was: neutrophilic leukocytes, 52.5 per cent; myelocytes, 20.5 per cent; eosinophilic leukocytes, 20.5 per cent; lymphocytes, 20.5 per cent, and plasma cells, 1 per cent. No micro-organisms could be found in the abscesses.

**CASE 5.—Diphtheritic Colitis in a Case of Chronic Myelogenous Leukemia with Terminal Acute Exacerbation.**—An Italian, 38 years of age, was well until April, 1930, when he was bothered by an intermittent pain in the left sacro-iliac region and by great fatigability. In June, he noticed a mass in the left hypochondrium. His condition was diagnosed as myelogenous leukemia, and roentgen treatment was applied over a period of four months. In all, twenty-five treatments were given over various areas, one-third erythema dose each. The white count, which on admission was 103,500, fell to 26,000 and then rose again to 60,000 to remain stationary. The last treatment was given on October 30, and the patient went home. Soon after this last treatment, he started to have a slight intermittent fever. The temperature went up to 100.2 F. He grew gradually weaker. The red count now was 4,500,000, but dropped within the next two weeks to 1,500,000, while the hemoglobin diminished from 70 to 45 per cent. A white count done two days prior to death revealed 41,950 cells, of which 81 per cent were myeloblasts, 3 per cent promyelocytes, 3 per cent myelocytes, 12 per cent neutrophils, 2 per cent basophils and 1 per cent lymphocytes. There was 1 normoblast to 200 white cells.

**Anatomic Diagnosis:** The anatomic diagnosis was hyperplasia of the cervical, axillary, inguinal, tracheobronchial, peri-aortic, peribiliary and peripancreatic lymph nodes; a huge splenic tumor (1,660 Gm.); moderate enlargement of the liver (2,230 Gm.); petechial hemorrhages in the mucosa of the lips, skin, epicardium, endocardium, pleura and lungs, and diphtheritic colitis. The mucosa of the transverse colon was raised to form soft, reddish-gray plaques, 5 mm. in diameter. Near the splenic flexure and extending through the descending and sigmoid colon into the rectum the mucosa was transformed into a 3 mm. thick, dirty gray, adherent membrane.

**Histologic Observations:** The bone marrow was myeloblastic. Scattered between the large, round, oxydase-positive cells, which were often found in the stage of mitotic division, were a few neutrophilic myelocytes, large orthochromatic normoblasts and very few intact megakaryocytes. The lymph nodes showed a similar picture, and in the liver the portal capillaries and the portobiliary septums

contained a great many myeloblasts and a few neutrophilic myelocytes. In the splenic pulp, however, there were decidedly more neutrophilic myelocytes, and there were also many nucleated red cells. In the inflamed colon, polymorphonuclear leukocytes were so numerous that one would not think that the sections came from a case of myeloblastic leukemia (fig. 2). They formed dense accumulations underneath the membrane of necrotic tissue. Myeloblasts and myelocytes were absent, and it was only in the edematous submucosa that a few of these cells could be detected.

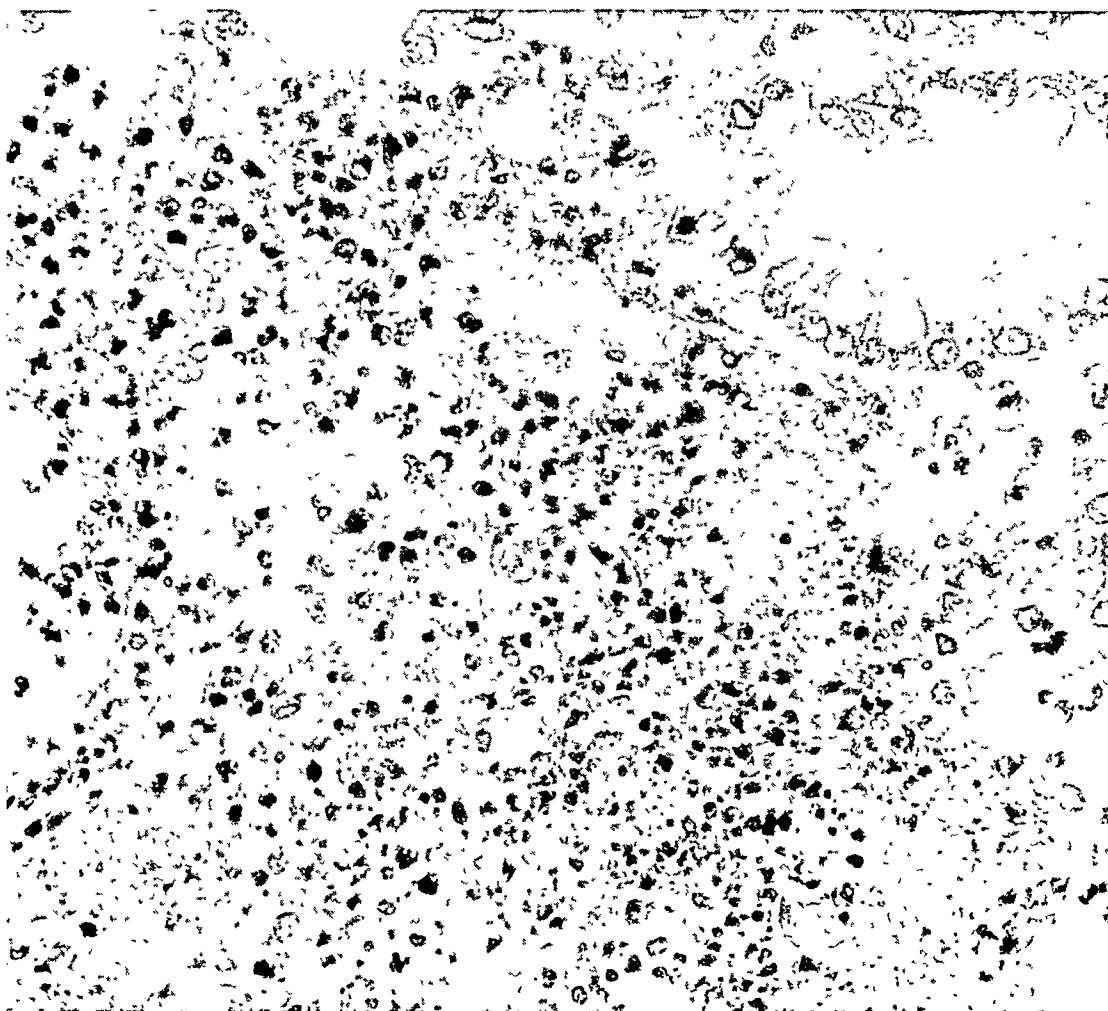


Fig. 2 (case 5).—Diphtheritic colitis. Note the accumulation of mature leukocytes underneath the necrotic membrane;  $\times 600$ .

*CASE 6.—Diphtheritic Tonsillitis and Laryngitis and Confluent Bronchopneumonia in a Case of Acute Stem Cell Leukemia.*—A white man, aged 35, stated that about six weeks before admission to the hospital he caught a severe cold which he tried to abort by sweating. Ever since, cough and profuse expectoration had persisted. Five days before admission, severe pains in the chest developed. During the last two weeks his temperature often went up to 103 F. The physical findings consisted of enlargement of the tonsils, pharyngitis with much mucopurulent secretion, a rough pleural rub over the base of the right lung and a pulse rate

of 110. The temperature on admission was 102 F. The patient died three days after entrance.

The erythrocyte count was 2,340,000; the hemoglobin content was 50 per cent. The white blood cell count was 19,300. Stem cells (cells with an ample, light blue cytoplasm, which is often vacuolated and is free from oxydase granules, and round nuclei, sometimes indented, containing a fine chromatin net and two or three large nucleoli) numbered 95 per cent; promyelocytes, 1 per cent; neutrophilic leukocytes, 1 per cent, and lymphocytes, 3 per cent. One nucleated red cell per hundred white cells was found. Anisocytosis and poikilocytosis were present.

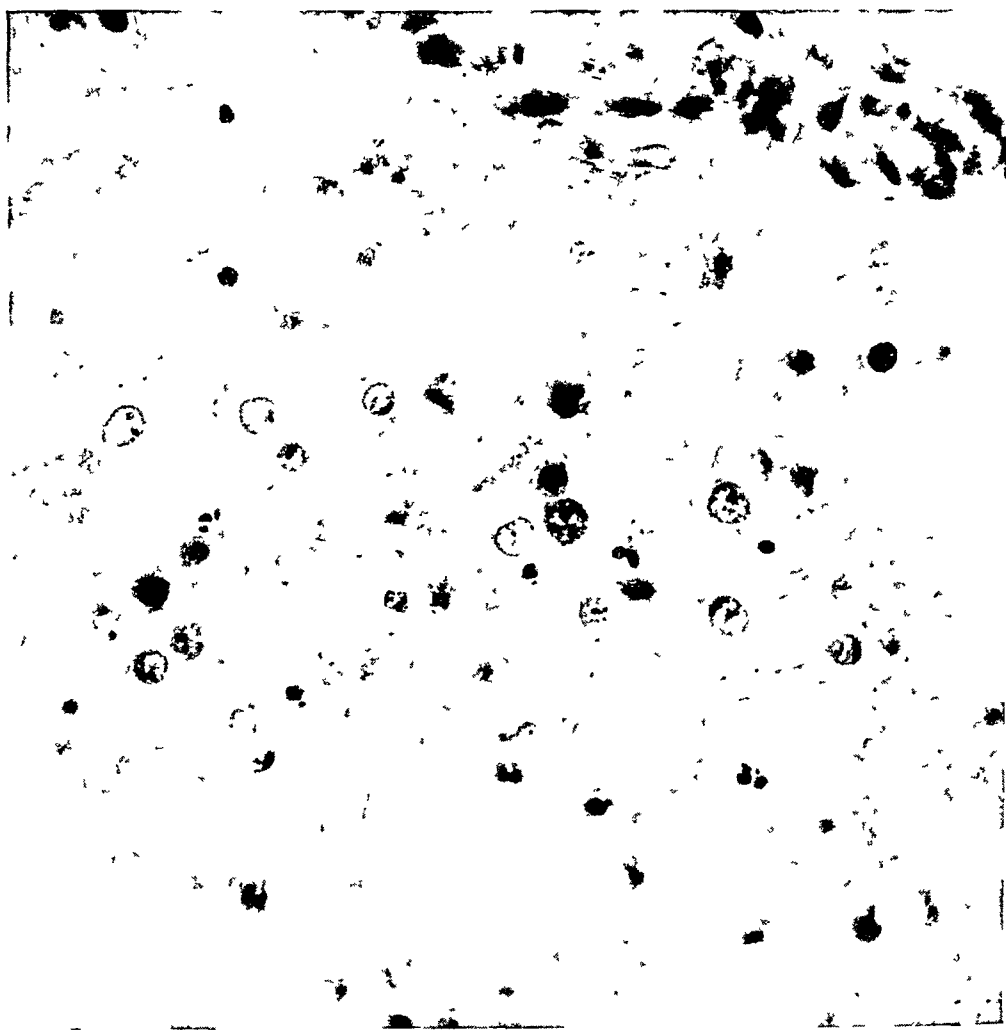


Fig. 3 (case 6).—Pneumonic area The exudate contains single stem cells and a few small and degenerated leukocytes;  $\times 600$ .

**Anatomic Diagnosis:** The anatomic diagnosis was petechial hemorrhages in the skin, epicardium, pleura, renal pelvis and colon mucosa; slight enlargement of the spleen (205 Gm.); moderate enlargement of the liver (2,360 Gm.); enlargement of the suprarenal glands (24 Gm.); enlargement of the lymph nodes up to 30 mm. in greatest diameter (the nodes were soft and light purple-gray with darker red areas); diphtheritic tonsillitis and laryngitis; edema of the epiglottis; confluent bronchopneumonia in both lungs, especially the right; severe anemia and hydremia; herpes of the left ear.

**Histologic Observations:** The bone marrow was very cellular and was composed mainly of large round cells with pale round nuclei and narrow rims of homogeneous, basophilic cytoplasm. The nuclei contained two or three distinct basophilic nucleoli. Many cells had two nuclei. Mitoses were numerous. The oxydase reaction was negative. There was an occasional cell with purple-pink granulation and a round nucleus. There was a single smaller cell with coarse oxyphilic granulation. A few polychromatophilic normoblasts, plasma cells and mast cells completed the picture. In some places there were small, sharply separated lymph follicles composed of small lymphocytes.

The cells described in the bone marrow predominated also in the liver and in the spleen, while in the lymph nodes they were less numerous and were mixed with small lymphocytes and plasma cells. The medulla in the suprarenal glands was densely infiltrated by the indifferent round cells, which also filled the capillaries of the reticular zone of the cortex.

The tonsils consisted of lymphadenoid tissue, and there were no abnormal elements. The surface was diffusely necrotic, and the necrosis extended in places deep into the tissue. It contained an enormous number of streptococci, and underneath it there were no reactive changes, and oxydase-positive cells were completely absent. The larynx showed a similar picture, and here, too, the complete lack of reaction about the necrosis was striking. The capillaries and small veins were occluded by plugs of fibrin. In the areas of consolidation of the lung, the alveoli were filled by an exudate that consisted chiefly of fibrin threads. In some of the alveoli, the fibrin was scanty, and in its meshes were found a few large round cells with one or two pale nuclei and narrow rims of basophilic, oxydase-negative cytoplasm. Here and there a small neutrophilic leukocyte with a shrunken and broken up nucleus and poorly defined oxydase-positive granulation was visible (fig. 3). The alveolar exudate contained many streptococci. From the cardiac blood a pure culture of hemolytic streptococci was obtained.

**CASE 7.—Hemorrhagic Fibrinous Pericarditis in a Case of Acute Myelogenous Leukemia.**—A colored woman, aged 53, complained that during the last year she had lost 60 pounds (27.2 Kg.), and that she had been suffering from progressive weakness. Since three weeks before admission she felt an aching and stitching epigastric pain, radiating to the back. She vomited from two to four times a day one-half cup of bright red clotted blood, and her stools were dark red or black. She had a history of malaria, an infra-umbilical laparotomy, two miscarriages and six normal deliveries.

The temperature was 97.2 F.; the pulse rate was 118, and the respiratory rate, 36. The blood pressure was 118 systolic and 80 diastolic. The eyes were fixed. There was slight cervical lymphadenopathy. The lungs showed decreased expansion on the left side; there were suppressed breath sounds and an absence of tactile fremitus in the left lower axillary region. A systolic murmur was heard over the apex of the heart. The liver extended four fingerbreadths below the costal arch. In the left hypochondrium, a firm mass was felt, which was believed to be the spleen. There was tenderness on deep palpation over the upper part of the abdomen. The patellar reflexes were absent.

The erythrocyte count was 2,400,000; the hemoglobin content was 70 per cent. The white blood cell count was 90,000, with myeloblasts 5.2, promyelocytes 44, myelocytes 5.2, neutrophils, 41.6, eosinophils 0.4, basophils 1.6 and lymphocytes 2 per cent. There were 8 normoblasts per hundred white cells.

The patient died three days after admission.

**Autopsy:** The spleen showed marked enlargement (440 Gm.). It was soft, and the pulp was light purple-red without structural markings. The liver showed



moderate enlargement (2,225 Gm.). There was swelling of the peripancreatic, peri-aortic and peribiliary lymph nodes. The largest nodes measured 5 cm. in diameter. The marrow of the femur was reddish gray and moderately firm. There were syphilitic aortitis with insufficiency of the aortic valve, eccentric hypertrophy of the heart (450 Gm.), verrucous endocarditis of the mitral valve, and hemorrhagic-fibrinous pericarditis. The pericardial sac contained 300 cc. of a cloudy, blood-stained fluid. The surface of the heart was covered by a soft, loosely adherent, light pink-gray membrane with distinct villi. The enlarged pericardial sac caused compression of the left lower pulmonary lobe. There was superficial necrosis of Peyer's patches of the lower ileum. A nodose goiter was present.

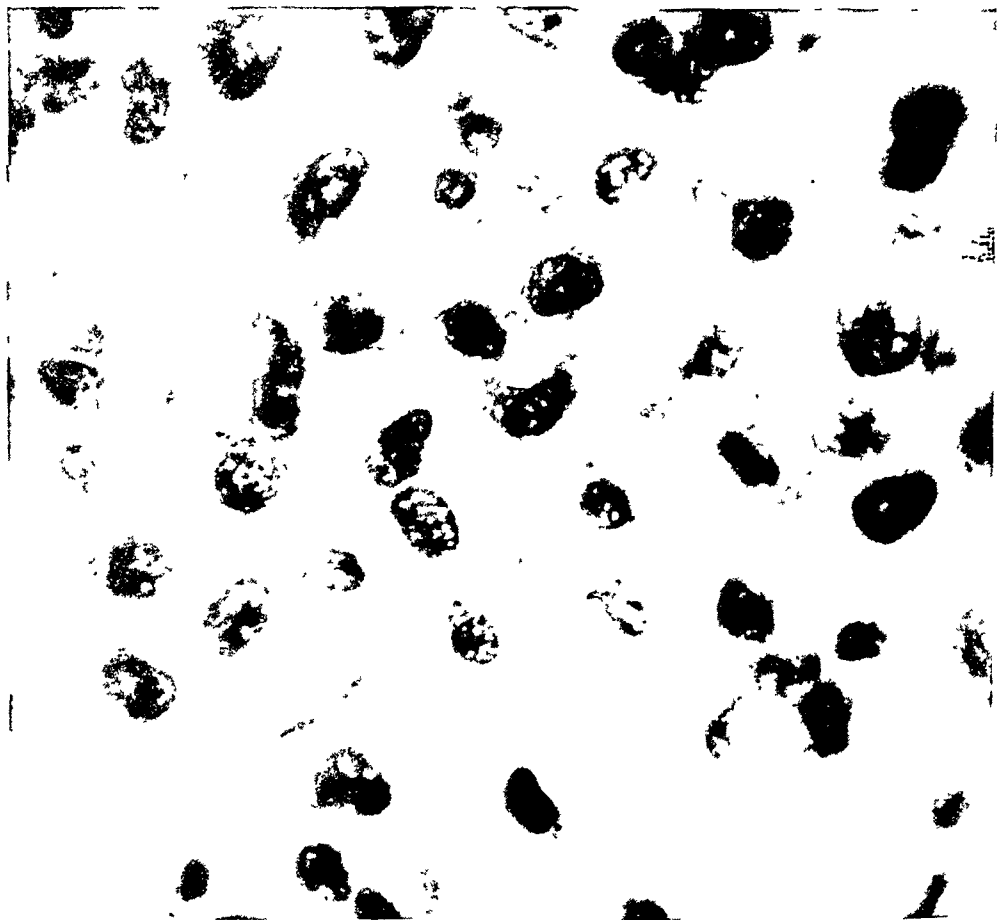


Fig. 4 (case 7).—Cytoplasmic syncytium in the epicardium; beginning condensation of the cytoplasm about the nuclei;  $\times 1,200$ .

**Histologic Observations:** The bone marrow was very cellular and showed active granulopoiesis with 43 per cent myelocytes and 9.8 per cent mature neutrophilic leukocytes. There were only few oxyphilic myelocytes and leukocytes, but many foci of erythropoiesis composed of erythrogonia, erythroblasts and normoblasts. In addition to these cells, there were 5 per cent plasma cells, 0.8 per cent monocytoïd elements and 0.6 per cent megakaryocytes. The splenic pulp was exceedingly cellular, the sinuses were hardly discernible, and the follicles were reduced to small perivascular accumulations of lymphocytes. Neutrophilic myelocytes were by far predominating. Scattered between them were small groups of myeloblasts, many orthochromatic normoblasts, few oxyphilic granulocytes and plasma cells.

The portal capillaries were stuffed by young neutrophilic myelocytes. There were also small groups of myeloblasts and orthochromatic normoblasts. The periportal tissue was infiltrated by myelocytes, lymphocytes and plasma cells. The abdominal lymph nodes contained numerous neutrophilic myelocytes, few oxyphilic myelocytes and single young and mature megakaryocytes. In the medullae of the kidneys were foci of granulopoiesis, which were much richer in oxyphilic myelocytes than were the granulopoietic foci in the other organs. The ulcerated plaques of the ileum showed many neutrophilic myelocytes and relatively few lymphatic elements. Underneath a superficial layer of necrosis, a narrow layer of mature neutrophils was found.

The cusps of the mitral valve revealed subendothelial circumscribed accumulations of mononuclear cells with oval or lobulated nuclei and ample, basophilic

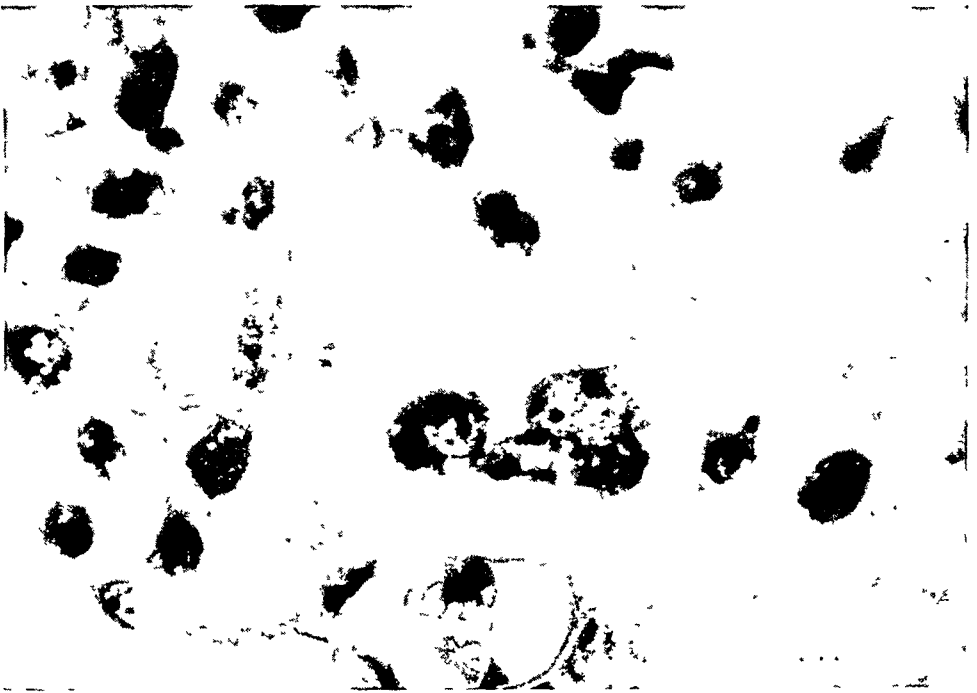


Fig. 5 (case 7).—Cytoplasmic syncytium in the epicardium. Three cells of myeloblast type are seen entering a pore;  $\times 1,200$ .

cytoplasm. In the thickened marginal portions, nodular areas stood out, which were composed of branched and stellate cells. These areas possessed a distinct affinity to the basic stains. Where the nodules protruded over the surface, they were covered by a homogeneous, hyaline material without bacteria.

The histologic observations in the inflamed epicardium were so unusual that they deserve a detailed description. The surface of the epicardium was covered by a layer of fibrin, which was thrown up to short and anastomosing villi. Between the villi there were a few ill-defined and degenerated granulocytes. Underneath the fibrin was a layer of cellular granulation tissue, which was very different from that commonly found in fibrinous inflammation with organization. It appeared as a spongy, protoplasmic syncytium with small, round, empty spaces. This syncytium contained round or oval nuclei with a distinct chromatin net, and the cytoplasm, which stained pale blue after Giemsa, was more compact about the nuclei (fig. 4).

From this indifferent, mesenchymatous syncytium two strains of cells developed. First, the cytoplasm around the nuclei became deeply basophilic, the chromatin content of the nuclei decreased, and one or two nucleoli become visible. Between the deeply basophilic cytoplasm and the rest of the syncytium a narrow crevice was formed, and the cell lost its connection with the protoplasmic net to enter one of the round, empty spaces previously described (fig. 5). The cytoplasm then contained fine, purple-gray granulation. The second strain of cells was characterized by progressing condensation of the chromatin until the nuclear structure was completely obscured. At the same time, the cytoplasm, which at first was deeply

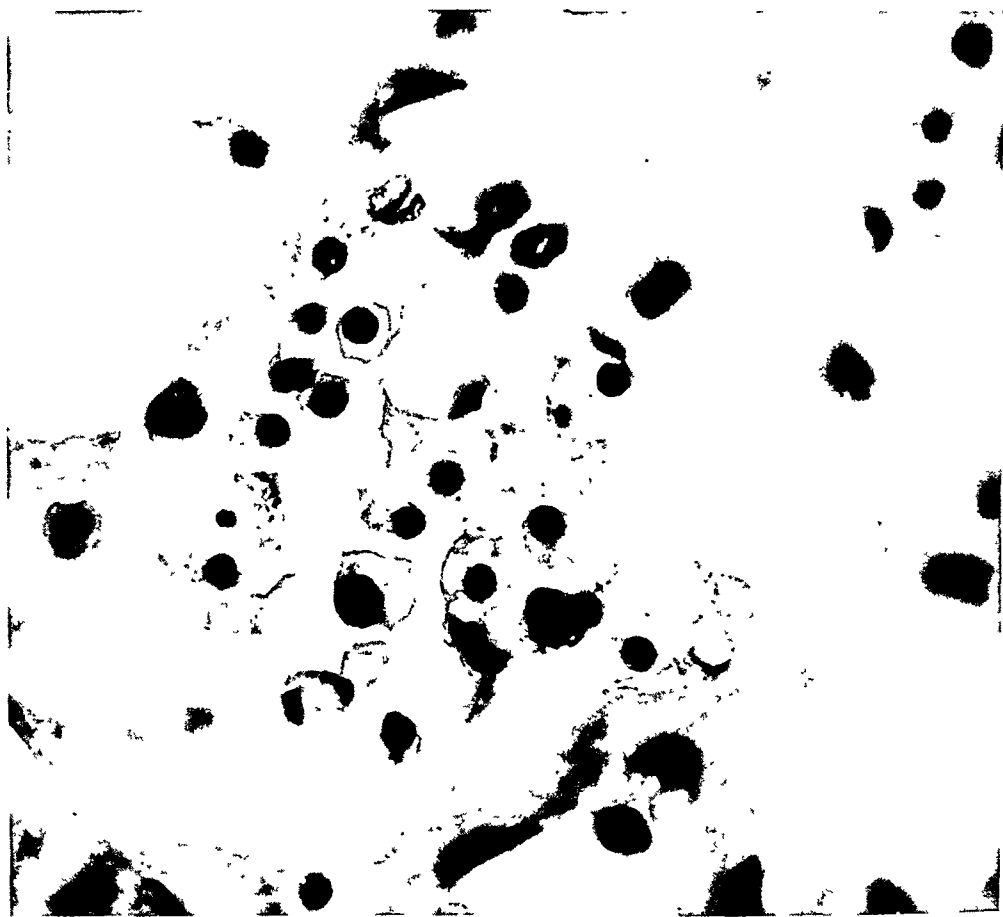


Fig. 6 (case 7).—Cytoplasmic syncytium in the epicardium; a group of nucleated red cells in a pore of the syncytium;  $\times 1,200$ .

basophilic, assumed a purple-red color which gradually changed to a bright brick red. Here and there one saw bright brick red droplets around the nucleus (fig. 6). These cells, which varied in size, also were discharged into the empty spaces. Where the protoplasmic syncytium approached the myocardium, the picture was somewhat different. The cytoplasm seemed to produce fine, parallel fibrils, which gradually filled the empty spaces. There was a moderate number of wide capillary blood vessels lined by flat endothelium and containing many nucleated cells, which did not show any relations to the endothelium. The myocardium was free from interstitial infiltrations. The histologic picture of the syphilitic aortitis was not different from that commonly found. There was much scarring of the media.

CASE 8.—*Bilateral Pyelitis and Pylonephritis and Recent Fibrinous Pericarditis in a Case of Chronic Myelogenous Leukemia.*—A white man, 48 years of age, an iron molder, felt well until five and a half years ago when he noticed languor and tired feeling after work. At times he was exhausted to the point that he was unable to move for from three to four hours. Shortly afterward headaches and head noises appeared that were relieved only by sleep. Six months later he discovered an enlargement of the left side of the abdomen. A physician was consulted, who prescribed irradiation of the spleen. After this treatment, all the symptoms subsided, and the patient was able to return to work. With roentgen treatment given every three months the patient did fairly well, but later the symptoms reappeared, and he went to the hospital.

On physical examination, the spleen was found extending down to the umbilicus, and the liver was palpable and very firm. The erythrocyte count was 2,560,000; the hemoglobin content was 60 per cent. The white cell count was 185,000, with a predominance of myelocytes. Treatment with the x-rays was again instituted, and the white count went down to 94,000, but soon returned to 192,000. The patient went home for a week and returned with marked weakness and severe pains in the abdomen. The erythrocyte count was 1,820,000, with hemoglobin 40 per cent. The white cell count was 31,000, with myeloblasts 24, promyelocytes 9.4, myelocytes 27, neutrophils 32.6, eosinophilic myelocytes 1.4, eosinophilic leukocytes 0.4, basophils 1.2 and lymphocytes 0.4 per cent. One day before the patient died, a definite pericarditic rub was heard. There was no rise in the temperature, and the patient expired quietly after an illness of five and a half years.

Anatomic Diagnosis: The anatomic diagnosis was as follows, large tumor of the spleen (1,050 Gm.); marked enlargement of the liver (3,460 Gm.); slight swelling of the peripancreatic, mesenteric, peri-aortic, inguinal and cervical lymph nodes; light yellow-green medullary bone marrow; eccentric hypertrophy of the heart (450 Gm.); fatty degeneration of the myocardium; recent fibrinous pericarditis; bilateral pyelitis; abscesses in the medulla in both kidneys (both renal pelves contained several light yellow, mulberry-shaped concretions up to 2 mm. in diameter); bilateral hydrothorax; hydropericardium, and ascites.

Histologic Observations: The bone marrow was very cellular and contained numerous myeloblasts, promyelocytes, neutrophilic and oxyphilic leukocytes and megakaryoblasts. The latter resembled overgrown myeloblasts. Eosinophilic myelocytes were relatively scanty. Erythropoiesis appeared in small foci of erythrogenia, erythroblasts and normoblasts. About 1 per cent of the cells was a mature megakaryocyte. The hepatic cells of the acinar centers were necrotic, while the Kupffer cells were filled by iron granules. Around the necrotic areas, much fat had accumulated in the hepatic cells. The portal capillaries contained a great many nucleated cells, chiefly neutrophilic myelocytes, then myeloblasts, oxyphilic granulocytes, normoblasts and few erythroblasts and erythrogenias. The periportal septums showed only a few loosely scattered myelocytes, myeloblasts and eosinophils.

In spite of the great cellularity of the splenic pulp, the reticulum was very prominent. The pulp was composed of an enormous number of oxydase-positive cells, among which promyelocytes and myeloblasts predominated. There were also many oxyphilic myelocytes and leukocytes and normoblasts, the latter varying considerably in size. Mature neutrophils were scanty. Here and there a young or fully developed megakaryocyte was found. The follicles had disappeared. The reticular cells were filled by iron pigment. The abdominal lymph nodes contained a varying number of myeloid cells. In the peribiliary nodes, the myeloid tissue was restricted to the interfollicular areas, and the follicles were still visible. In

the peri-aortic lymph nodes, the lymphatic tissue had been completely substituted by granulopoietic tissue. The inguinal lymph nodes were rich in young and adult megakaryocytes.

The abscesses in the medulla of the kidney showed diffuse coagulation necrosis with clumps of degenerated polymorphonuclear leukocytes and nuclear debris. There was no well defined zone of demarcation save for a narrow rim of leukocytes and small round cells. The wall of the pelvis was densely infiltrated by various types of immature blood cells, most of which were oxydase-positive. Some of the oxydase-positive cells were large, distinctly larger than in the other organs. Their shape was irregular, and blunt processes projected from the surface. The nuclei were round and pale with fine chromatin granules. A few of the oxydase-positive cells were flat and drawn out. The fibrocytes between the infiltrating cells were very large; their cytoplasm stained basophilic and was finely vacuolated. The



Fig. 7 (case 8).—*A* shows a large fibrocyte in the epicardium. Note the deep basophilic coloration of the cytoplasm. The large pale nucleus with the distinct nucleoli resembles the nucleus of a hemocytoblast;  $\times 1,200$ . *B* shows the fibroblast after it has become separated. The cytoplasm contains a fine, oxydase-positive granulation, which is not visible in the halftone picture;  $\times 1,200$ .

nuclei were pale and contained prominent nucleoli. Between these cells there were single megakaryocytes and oxyphilic myelocytes.

The pericarditis was more recent than in case 7. The fibrin that covered the surface formed slender trabeculae, and near the base desquamated mesothelial cells could be seen. Only a few immature white blood cells were present. The epicardium was loosened, and the fibrocytes were very prominent as large, branched elements with a basophilic, often finely vacuolated cytoplasm and round nuclei containing either a single angular or several round nucleoli (fig. 7 *A*). The loosened connective tissue with the large fibrocytes formed a net in the meshes of which accumulations of promyelocytes, oxyphilic and neutrophilic myelocytes and single lymphocytes and plasma cells were found. Neutrophilic leukocytes, too, were present, most of which had deeply lobulated nuclei and very indistinct granulation.

There were also the large cells with the blunt protoplasmic projections described in the renal pelvis (fig. 7 B). In the Giemsa sections, the cytoplasm assumed a bluish-gray color, and very fine, purple-pink granules could be differentiated. The nuclei were round or oval and contained fine chromatin granules and two or three distinct nucleoli. The capillaries of the epicardium were dilated and contained a varying number of immature blood cells. Though the endothelium was swollen, it remained pale and did not reveal an increased affinity to the basic stain. About the capillaries there were large branched cells, which seemed to be connected with the swollen fibrocytes.

CASE 9.—*Chronic Pneumonia in a Case of Chronic Myelogenous Leukemia.*—A white man, aged 80, was under the care of an outside doctor and was in the hospital for only four and a half hours. Six years before admission he had been operated on for ulcers of the stomach, and five years before admission he had a gas anesthesia for extraction of teeth from which he was resuscitated with great difficulty. One and a half years before admission he began to bleed from the mouth and the rectum, and a small cut on the finger would bleed for a day or two.

The temperature was 94.2 F.; the respiratory rate, 24; the pulse rate, 60. The heart was enlarged, and the tones were very weak. The liver and the spleen were markedly enlarged. The urine contained two plus albumin and many hyaline and granular casts.

The erythrocyte count was 2,040,000, with a hemoglobin content of 20 per cent. The white cell count was 320,000, with myeloblasts 1, promyelocytes 7, metamyelocytes 4.5, neutrophils 67.5, eosinophils 2.5, basophils 8 and lymphocytes 9.5 per cent. There were 14 normoblasts per hundred white cells, also marked anisocytosis and hypochromia.

Anatomic Diagnosis: The anatomic diagnosis was marked enlargement of the spleen (530 Gm.) and liver (1,980 Gm.); enlargement of the peri-aortic lymph nodes up to a diameter of 18 mm.; dark red bone marrow; hypertrophy of the heart (525 Gm.); old gastrojejunostomy with two ulcers in the jejunum opposite the opening; healed peptic ulcer of the stomach with marked pyloric stenosis; chronic emphysema of the lungs and chronic pneumonia in the upper half of the right upper lobe, and arteriosclerotic contraction of the kidneys.

Histologic Observations: Microscopic examination of the bone marrow revealed great cellularity with numerous promyelocytes and neutrophilic and oxyphilic myelocytes and groups of myeloblasts. There were many areas of erythropoiesis and many megakaryocytes. The cords of the splenic pulp contained many myeloblasts, promyelocytes and a moderate number of neutrophilic and oxyphilic granulocytes. The abdominal lymph nodes showed moderate myeloid metaplasia with a predominance of young neutrophilic myelocytes. In contrast to the spleen and the abdominal lymph nodes, the liver showed only a few small intra-capillary granulopoietic foci. The centers of the acini were necrotic. The histologic picture of the jejunal ulcers was that of chronic peptic ulcers with broad zones of fibrinoid necrosis passing into a moderately cellular granulation tissue containing a few neutrophilic myelocytes. In the thickened submucosa there were larger groups of myelocytes.

The carnified area in the right upper pulmonary lobe was made up of alveoli, some of which were filled by hyaline fibrin, while others contained cellular granulation tissue. In many places, the organization of the exudate had advanced so far as to obscure the alveolar outlines. Between the fibrocytes occupying the alveolar spaces were groups of from ten to twenty-five large round cells with round, light-

stained nuclei and fine, purple-pink granulation. Oxydase stain demonstrated numerous granules in these cells. Some of the cells were undergoing mitotic division. The other organs, pancreas, suprarenal glands, kidneys, etc., were free from myeloid tissue.

CASE 10.—*Gumma of the Lung and Metastases of a Melanoblastoma of the Eye to the Liver in a Case of Acute Myeloblastic Leukemia.*—A Rumanian, aged 74, had his left eye removed for a pigmented tumor. A year later he entered the hospital with the complaint of severe pains in the mouth. The temperature was 99.6 F.; the pulse rate, 96; the respiratory rate, 20. The blood pressure was 120 systolic and 50 diastolic. In addition to severe gingivitis, clinical examination revealed a brownish-red macular and papular rash over the chest and abdomen, hyperresonance of the lungs with numerous râles, and enlargement of the heart to the left. Smears taken from the mouth showed many streptococci, but no fusiform bacilli or spirochetes. The Kahn and Wassermann reactions were three plus.

The erythrocyte count was 2,670,000; the hemoglobin content was 50 per cent. The white cell count was 50,800, with myeloblasts 80, neutrophils 4, monocytes 1 and lymphocytes 15 per cent. Dental and oral hygiene improved the condition of the mouth, but the rash on the body became widely spread. The course was afebrile but marked by rapidly progressing weakness. The patient died after a stay in the hospital for sixteen days. The duration of the illness had been four and a half weeks.

Autopsy: Post mortem, numerous slightly elevated, firm nodules, from 1 to 4 mm. in diameter, were found in the skin of the neck and the upper half of the chest. The nodules, which seemed to be located in the cutis, had the same color as the skin, namely, a light gray-brown. In the left cubital fossa and over the volar aspect of both arms, many pinhead-sized hemorrhages were present. The left eye was replaced by a prosthesis. In both groins and axillae, firm, discrete lymph nodes, ranging in size up to that of a chestnut, could be felt. The teeth were in poor condition, and the mucosa of the mouth was pale. The heart weighed 510 Gm. Underneath the epicardium and endocardium of both ventricles, there were many flat, grayish-white, discrete and confluent nodules, which measured from 1 to 5 mm. in diameter. Similar nodules could be seen in the wall of the pulmonary conus. Syphilitic aortitis was present, involving the aortic valve and causing moderate insufficiency of the valve. There was a small aneurysm of the descending aorta below the origin of the left subclavian artery. Emphysema of the lungs was present. In the center of the left lower lobe there was a sharply circumscribed, ovoid nodule 25 by 12 by 10 mm. in diameter. The nodule consisted of an opaque, yellow-gray center and a dense, fibrotic, deep-gray periphery. It was closely adjacent to, but separated from, a bronchus of the third order.

The spleen weighed 500 Gm. The liver weighed 1,670 Gm. The surface was studded by many whitish, dark brown and black nodules, which ranged from the size of a pinpoint to a diameter of 40 mm. The pigmented nodules were larger and more numerous than the white ones. On the sectioned surface, the same nodules were visible and occupied about one fifth of the parenchyma. The intervening hepatic tissue was gray-brown. There were hemorrhages in the mucosa of the lower part of the ileum and in the ascending colon. There were small whitish nodules in the cortex in both kidneys. The abdominal lymph nodes were enlarged and light purple. The bone marrow was light red-gray and soft. The brain weighed 1,140 Gm. It was very pale and moist. The retrobulbar tissue of the left side was free from tumor tissue.

**Histologic Observations:** In the bone marrow, the predominating type of cell was a large lymphoid round cell with ample, homogeneous, basophilic cytoplasm. The nuclei were round or slightly indented and contained a few small chromatin granules and several distinct nucleoli. A few of the cells showed fine, purple-pink granulation. There were numerous mitoses. Scattered between these cells were small foci of erythropoiesis with erythrogonias, erythroblasts and normoblasts. Other cell forms were scanty. There were few mature oxyphilic leukocytes, single plasma cells and occasional neutrophilic leukocytes with shrunken nuclei. The cords of the splenic pulp were uniformly composed of the large lymphoid cells found



Fig. 8 (case 10).—Artery near the gumma in the lung. In the thickened intima, numerous immature blood cells are to be seen; elastic stain;  $\times 300$ .

in the bone marrow. The follicles were small and lymphocytic. The large lymphoid cells had also replaced most of the lymphatic tissue of the abdominal and inguinal lymph nodes, the secondary follicles being reduced to small groups of lymphocytes.

The dark brown and black nodules of the liver consisted of elongated spindle-shaped cells, which were filled by dark brown pigment granules. The cells formed small alveoli, which were surrounded by a scanty stroma. In addition to the elongated cells, there were single large elements of irregular shape, which were so densely packed by pigment granules that the nucleus was completely obscured. The white nodules showed an entirely different picture. They were formed by the



basophilic round cells previously described, about one fourth of which gave a positive oxydase reaction. The pigmented and nonpigmented nodules occasionally fused together, and the two different types of cells mingled with each other. Lymphoid cells and pigmented cells could be seen lying side by side without interfering with their respective proliferations. The lymphoid cells were also present in the portal capillaries and in the periportal septums. Many of the Kupffer cells contained melanotic pigment, which was also found in some of the hepatic cells. An interesting finding were cylinders of melanotic pigment in the perivascular lymph spaces and in the intercellular bile capillaries.

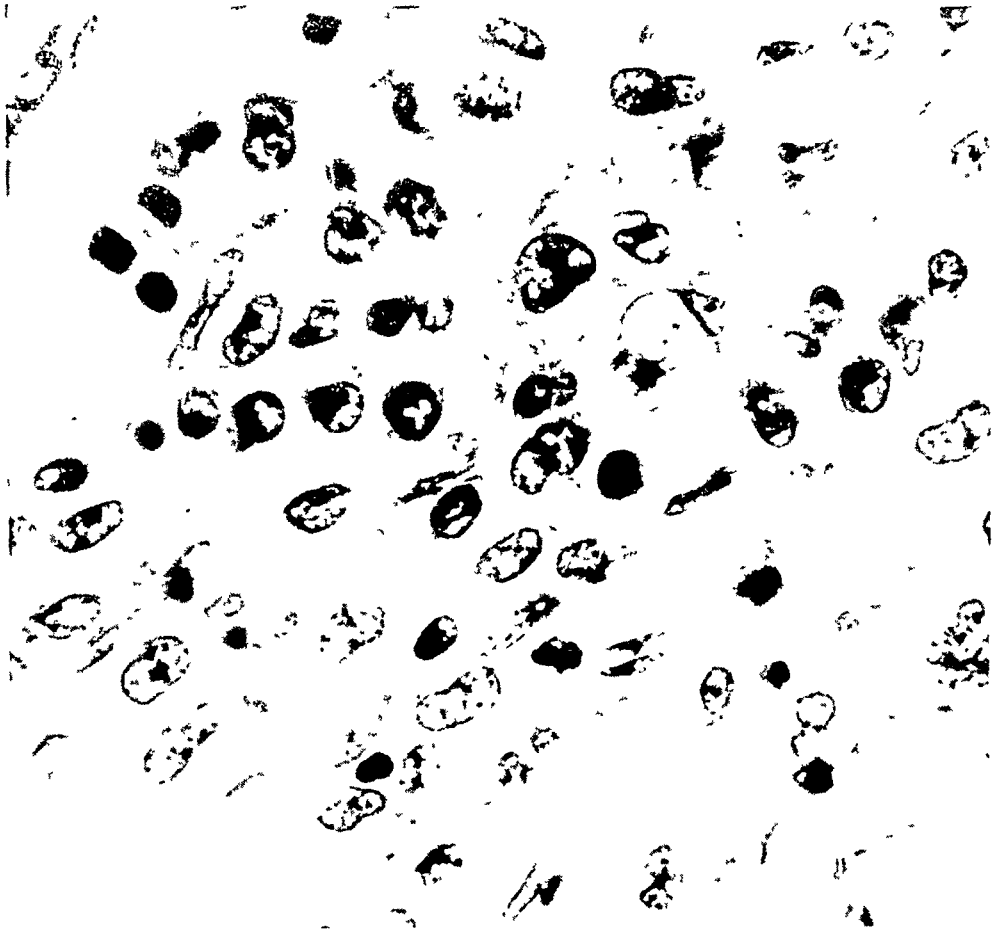


Fig. 9 (case 10).—Group of young myelocytes in the intima of the artery shown in figure 8. Note the reticulum between the myelocytes;  $\times 1,200$ .

The nodules in the heart and skin were composed of lymphoid round cells, about half of which were oxydase-positive. In the skin, the cells were located in the papillary and reticular layer of the cutis and were separated from the epidermis by a band of hyaline tissue. The cells often accumulated about sebaceous and sweat glands. The nodules in the kidneys, too, were made up of these cells.

The nodule in the left lobe of the lung contained a necrotic center, which was surrounded by a capsule of connective tissue with small accumulations of lymphocytes and plasma cells. In the necrotic area, the outlines of blood vessels with much thickened walls and occluded lumen were discernible. Bielschowsky-Maresch silver impregnation demonstrated many argentaphil fibrils in the necrotic areas,

which, according to Coronini, is characteristic of a gumma. Adjacent to and extending into the node there were several small veins and arteries the intima of which was much thickened, and the adventitia and media of which were infiltrated by small round cells. The intima appeared very cellular, but the cells were of different character as compared with those in the media and adventitia (fig. 8); they possessed ample, basophilic cytoplasm and round or bean-shaped nuclei. The cytoplasm often contained fine, purple-pink granulation, especially near the nucleus. These cells spread apart the connective tissue fibrils of the intima, which became transformed into a reticulum. The fibrocytes increased in size, and their cytoplasm assumed basophilic coloration, while the nuclei resembled the nuclei of the cells in the meshes of the reticulum (fig. 9). There were no other changes in the lung, except an occasional group of large lymphoid round cells about a small blood vessel. The histologic picture of the aorta was that of typical syphilitic aortitis with advanced scarring.

#### COMMENT

Of the three patients with lymphatic leukemia, two had responded to an acute infection like normal persons, namely, by the production of a leukocytic exudate. The unusually severe reaction to the anemic splenic infarct in the second case could be explained by the presence of streptococci in the infarcted area. The port of entrance of the streptococci was apparently the sore throat from which the patient had been suffering shortly before his death. In these two cases, the bone marrow contained myelocytes, and especially in the second observation the myelocytes were found in active proliferation and originating from undifferentiated cells about the blood vessels. In chronic lymphatic leukemia, small groups of myelocytes are usually present in the bone marrow (Naegeli, Helly<sup>16</sup>), and it is not uncommon to find single immature granulocytes in the peripheral blood in these cases (Priesner<sup>17</sup>). According to Piney,<sup>18</sup> the myeloid tissue surrounds the accumulations of lymphatic cells much as it surrounds the metastases of malignant tumors to the bone marrow. In this observation, Piney saw a support for his conception that leukemia belongs to the true tumors. Banti<sup>19</sup> distinguished four stages of the bone marrow changes in lymphatic leukemia, namely, hyperemia, hematopoiesis, nodular proliferation and diffuse proliferation of the lymphatic tissue. Myeloid tissue, however, is found not only in the third but also in the fourth stage, and it seems to me that the active proliferation of granulopoietic tissue in a diffusely lymphatic bone marrow rather speaks against the tumor theory. In acute lymphatic leukemia, too, foci of myelocytes are found in the bone marrow.

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16. Helly, K.: *Leukaemien*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1927, vol. 1, pt. 2, p. 1015.

17. Priesner, E.: *Wien. klin. Wchnschr.* **43**:170, 1930.

18. Piney, A.: *Am. J. M. Sc.* **169**:691, 1925.

19. Banti: *Le leucémie*, *Atti d. VIII. riun. d. Soc. ital. di patol.*, 1913.

The granulopoietic tissue was not restricted to the bone marrow. In the first case, myelocytes could be detected also in the splenic pulp, while in the second case they were observed in the spleen, in the liver and, particularly, in the abdominal lymph nodes, the sinuses of which contained numerous streptococci. Naegeli, who described myelocytes in the lymph nodes in cases of lymphatic leukemia, considered them as compensatory for the lymphatic replacement of the bone marrow. Thus, in the first two observations, sufficient myeloid tissue was present to supply granulocytes for a leukocytic exudate.

The third patient, who had subacute aleukemic lymphadenosis changing later into the leukemic form, reacted differently to a complicating infection which was followed by streptococcic septicemia. Under the influence of the septicemia, the white count dropped within a few days from 53,000 to 850 and, save for the severe anemia, the blood picture resembled finally an agranulocythemia. About the necrotic lesions in the mouth there was not a single granulocyte, and the lymphatic elements were unable to compensate for the lack of leukocytic response. Nowhere in the body were there any myelocytes. In the bone marrow, only an occasional large oxyphilic leukocyte was found, and the lymph nodes showed a few myeloblasts. In the lymph nodes and in the spleen, the leukemic cells revealed very severe regressive changes, which caused a peculiar loosening of the structure of these organs. In the bone marrow and in the liver, the disintegration of the cells was much less marked. A. Schultz,<sup>20</sup> who described septicemia in a case of aleukemic lymphadenosis turning into the leukemic stage, was struck by the complete absence of mesenchymatous reaction in this case as compared with other cases of septicemia.

In two cases of myeloid leukemia complicated by acute infections, the immature granulocytes took little part in the local defense reactions, while mature granulocytes were abundant. It is interesting that the prostatic abscesses contained only 5.5 per cent myelocytes, while these cells were much more numerous in the interstitial infiltrations. The severe diphtheritic colitis in the chronic myelosis with terminal acute exacerbation was undoubtedly due to the intensive roentgen treatment. I have repeatedly seen these severe inflammations of the descending and sigmoid colon as the result of continued irradiation of the spleen. In spite of the 81 per cent myelocytes in the peripheral blood, mature granulocytes only were found underneath the necrotic membrane of the colon.

In this group of cases, too, there is one which is characterized by the lack of defense reaction. This case belongs to the type of leukemia that is usually called stem cell leukemia because the cells are so undiffer-

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20. Schultz, A.: *Krankheitsforschung* 8:206, 1930.

entiated that they cannot be identified with certainty. Since the organic changes resembled those found in myelosis, I have included this case among those of myeloid leukemia. The scarcity of myelocytes in the bone marrow readily explains the absence of granulocytes from the inflammatory lesions. In the tonsils and larynx there was practically no reaction to the necrosis, and in the pneumonic areas only a few stem cells had entered the alveoli.

In three cases of myeloid leukemia, fibrinous exudation with organization was found. The organizing granulation tissue produced blood cells, the origin of which could be studied best in the two cases of fibrinous pericarditis. In one case I have referred to a spongy syncytium located underneath the fibrinous membrane and giving rise to myelocytes and normoblasts. This syncytium answers to Hueck's<sup>21</sup> description of the most primitive form of mesenchymatous tissue. According to Hueck, the basic structure of the embryonic mesenchyma is a sponge composed of protoplasmic septums with nuclei in the knots, and of pores filled by tissue fluid. By differentiating fibrils on the free surface of the protoplasmic septums and keeping the pores open, reticular tissue is formed, while fibrillar connective tissue results from the filling of the pores by fibrils and ground substance. The production of the blood cells starts with condensation and increased basophilia of the cytoplasm about the nuclei. The cells retract from the rest of the syncytium and come to lie free in the tissue fluid that fills the pores. Normally, in postembryonal life, the basic structure of the mesenchyma is obscured because the pores are filled with some kind of differentiated ground substance. Even during the neoformation of connective tissue the syncytial structure remains invisible because the production of fibrils dominates the picture from the beginning. There exists apparently an important difference between the normal mesenchyma and the mesenchyma of the leukemic person, which manifests itself when the mesenchyma is irritated. Though the irritated mesenchyma in leukemia may produce collagenous ground substance, it also possesses the property to develop into myeloid tissue by passing through the stage of the undifferentiated protoplasmic syncytium. Granulocytes and erythrocytes differentiate side by side from this syncytium, and there seems to be an early common stage corresponding to Maximow's hemocytoblast.

In case 8, the pericarditis was early, and the process of organization was in its beginning. The irritated fibrocytes were very large with deeply basophilic cytoplasm. They differed from the fibrocytes commonly seen in inflammation by a peculiar nuclear differentiation with prominent angular nucleoli and by their transformation into abnormal, huge granulocytes. This skipping of the hemocytoblast stage during

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21. Hueck, W.: Beitr. z. path. Anat. u. z. allg. Path. 66:330, 1920.

the formation of granulocytes has been repeatedly described, and, especially in leukemia, specific and oxydase granules may appear in cell forms that are not yet separated. Stockinger<sup>22</sup> saw in this abnormal mesenchymal differentiation a disproportion between irritation and response.

One may question whether the large basophilic, granule-producing cells are fibrocytes and not histiocytes. It is true that the differentiation between these two types of cells, especially when irritated, may cause great difficulty, but it is the rich branching of the cells and their intimate relation to the ground substance that have induced me to consider them as fibrocytes.

Marchand's pupil, G. Herzog,<sup>23</sup> who advocated the local formation of granulocytes in inflammation from adventitial elements, described a case of uremic pericarditis with production of granulocytes in the sub-epicardial tissue. He depicted the formation of granules in large adventitial cells, but saw also immature granulocytes in the lumen of the dilated blood vessels. Since he did not give the blood findings or the histologic changes in the other organs, it is difficult to draw any conclusions from his case. I have examined a number of pericarditides, in particular the uremic form, but so far have been unable to find evidences of local granulopoiesis.

In the case of myelosis with chronic pneumonia, the extramedullary myelopoiesis was restricted chiefly to the spleen and abdominal lymph nodes. In the area of organization of the pneumonic exudate, groups of young myelocytes were scattered between the proliferating fibrocytes.

One of the most interesting observations is the combination of acute myeloblast leukemia with metastases of a melanoblastoma of the eye to the liver and a syphilitic granuloma of the lung. The combination of leukemia and malignant tumor is very rare (Hirschfeld,<sup>14</sup> Glückmann,<sup>24</sup> Lüder,<sup>25</sup> Marischler<sup>26</sup> and others), and it has been said that when a malignant tumor develops in a leukemic patient, the leukemic symptoms may regress (Zadek,<sup>27</sup> Marischler). In my case there was no interference between leukemia and tumor, and in the liver the two types of cells were found growing side by side.

The lesion in the lung was diagnosed as gumma because of its location in the center of the lower lobe, the vascular changes about the lesion, the presence of argentaphil fibrils in the necrotic area and the positive serologic reactions. The proliferated intima of the thickened

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22. Stockinger, W.: *Ztschr. f. d. ges. exper. Med.* **58**:777, 1928.

23. Herzog, G.: *Centralbl. f. allg. Path. u. path. Anat.* **31**:481, 1921.

24. Glückmann, cited by Hirschfeld.<sup>14</sup>

25. Lüder, cited by Hirschfeld.<sup>14</sup>

26. Marischler: *Wien. klin. Wchnschr.* **9**:686, 1896.

27. Zadek, cited by Hirschfeld.<sup>14</sup>

vessels near the granuloma contained groups of young myelocytes, and between these myelocytes the connective tissue was transformed into a reticulum. Here, too, I obtained the impression that the myelocytes were formed locally, and that the fibrocytes acted as their parental cells. Against immigration from the blood spoke the fact that the myelocytes were most numerous near the internal elastic membrane, and that the subendothelial layer was free from these cells.

#### CONCLUSIONS

From the histologic study of the inflammatory lesions found in ten cases of leukemia the following conclusions can be drawn:

Provided that some myeloid tissue capable of maturation is left, the leukemic organism reacts to an infection like a normal one. Even in a diffusely lymphatic marrow, the myeloid cells can proliferate, encroaching on the lymphatic tissue, when stimulated by an infection. The literature contains many observations dealing with the return of normal blood findings in leukemic patients during or after an intercurrent infection (Allacia,<sup>28</sup> Körmöczy,<sup>29</sup> Funk,<sup>30</sup> Naegeli,<sup>8</sup> Hirschfeld,<sup>14</sup> Dock<sup>13</sup> and others). These facts are difficult to reconcile with the conception that leukemia belongs to the malignant tumors. The restoration of a normal hematopoiesis in leukemia under the influence of an infection cannot be compared with the disappearance of an epithelioma of the skin over which erysipelas is spreading, since one is dealing not merely with a regression of the leukemic tissue but with the resumption of an exceedingly intricate function by the diseased organ.

If the granulopoiesis is completely exhausted, there is no defense reaction to an infection. I could not confirm the statements made by Dionisi<sup>11</sup> and Bickhardt<sup>12</sup> that the leukemic cells may take the place of the mature granulocytes, very few immature blood cells appearing in the inflamed area.

It is generally accepted now that the granulated cells of an exudate come from the blood, and any attempt that has been made to question Cohnheim's fundamental observation has been doomed to prompt refutation (Henke and Silberberg<sup>31</sup>). Extramedullary granulopoiesis, however, does occur under a variety of abnormal conditions, and though in some instances it may result from the colonization of immature bone marrow cells (Jaffé<sup>32</sup>), in the majority of the cases the cells are formed locally. It is beyond the scope of this article to enter into a discussion

28. Allacia, G. B.: *Clin. med. ital.* **12**:1, 1902.

29. Körmöczy: *Folia haemat.* **11**:297, 1911.

30. Funk, cited by Helly.<sup>16</sup>

31. Henke, F., and Silberberg, M.: *Klin. Wchnschr.* **11**:49, 1932.

32. Jaffé, R. H.: *Beitr. z. path. Anat. u. z. allg. Path.* **68**:224, 1920.

of the various theories on extramedullary myelopoiesis. I refer to the recent reviews by Maximow<sup>33</sup> and Lang.<sup>34</sup> Suffice it to say that in the normal organism the undifferentiated, potentially myelopoietic tissue is apparently located between the adventitial histiocytes about the smaller blood vessels and in the cellular reticulum of certain organs, where it is hidden because of its insignificant appearance (Maximow). The histiocyte and fibrocyte are unable to change into hemocytoblasts. In leukemia, however, the range of the cells that may develop into blood cells is much wider. In previous publications I have described the transformation of histiocytes into myeloid cells in cases of leukemia, and the observations on inflamed tissue have convinced me that also the fibrocyte may do so. Freund,<sup>35</sup> who studied the effect of x-rays on leukemic infiltrations of the skin, also came to the conclusion that the myeloid cells were derived locally from fibrocytes. On the other hand, I have been unable to find any evidences for a reversion of the leukemic cells into fibrocytes or for their transformation into other cells (macrophages). The modern literature contains many reports on such transformations obtained in vitro (Awrorow and Timofejewsky,<sup>36</sup> Timofejewsky and Benewolenskaja,<sup>37</sup> Verati,<sup>38</sup> Hirschfeld,<sup>39</sup> Hirschfeld and Rawidowicz,<sup>40</sup> Katsunuma,<sup>41</sup> Silberberg and Voit,<sup>42</sup> Bloom<sup>43</sup> and others). This discrepancy between the findings inside the body and in tissue culture shows again how careful one should be in drawing conclusions from observations made on explanted cells.

I have so far been unable to find morphologic evidences for a transformation of fibrocytes into lymphocytes. Since lymphadenoid tissue is present practically everywhere in the body, this question will be difficult to decide. In this connection it may be mentioned that, in lymphatic leukemia, infiltrations not seldom develop in cutaneous scars, especially those after herpetic lesions and burns (Halle<sup>44</sup>).

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33. Maximow, A.: Bindegewebe und blutbildende Gewebe, in von Möllendorff, M.: *Handbuch der mikroskopischen Anatomie des Menschen*, Berlin, Julius Springer, 1927, vol. 2, pt. 1.

34. Lang, F. J.: *Folia haemat.* **43**:95, 1931.

35. Freund, F.: *Virchows Arch. f. path. Anat.* **369**:501, 1928; *Klin. Wchnschr.* **7**:977, 1928.

36. Awrorow, P., and Timofejewsky, A.: *Virchows Arch. f. path. Anat.* **216**:184, 1914.

37. Timofejewsky, A., and Benewolenskaja, S. W.: *Virchows Arch. f. path. Anat.* **263**:719, 1927; *Arch. f. exper. Zellforsch.* **8**:1, 1929.

38. Veratti, cited by Gloor, W.: *Folia haemat.* **45**:267, 1931.

39. Hirschfeld, cited by Silberberg and Voit.<sup>42</sup>

40. Hirschfeld and Rawidowicz, cited by Silberberg and Voit.<sup>42</sup>

41. Katsunuma, S.: *Tr. Jap. Path. Soc.* **19**:258, 1929.

42. Silberberg, M., and Voit, K.: *Deutsches Arch. f. klin. Med.* **171**:110, 1931.

43. Bloom, W.: *Arch. f. exper. Zellforsch.* **11**:145, 1931.

44. Halle, H.: *Arch. f. Dermat. u. Syph.* **159**:238, 1930.

The microscopic findings in inflamed tissue again demonstrate the sharp separation between the lymphopoietic and the myelopoietic tissue. Never was I able to find any indications of a transformation of leukemic lymphatic cells into hemocytoblasts and granulocytes, and whenever in lymphatic leukemia the body mobilized granulocytes it resorted to the undifferentiated germinal tissue about the blood vessels. It is significant that in spite of the abundant proliferation of the lymphatic tissue the undifferentiated cells have retained their granulopoietic properties. As far as the inflammatory reactions are concerned, there is no difference between the acute and chronic leukemias, which speaks against the conception held by C. Sternberg, and others that these two diseases do not belong together.

#### SUMMARY

The histologic pictures of the inflammatory defense reactions in ten cases of leukemia are given. It has been shown that the type of response depends on the presence of myeloid tissue able to produce mature granulocytes. In the presence of such tissue, the leukemic patient reacts to an infection like a normal person, while in the absence of such tissue the leukemic cells are not able to compensate, and the alterative changes predominate as they do in agranulocythemia and aplastic anemia. It seems that for the leukemic organism von Möllendorff's conception of the myelopoietic potencies of the fibrocyte holds true.



# METABOLISM IN ACUTE MOLECULAR DEGENERATION OF STRIATED MUSCLE

## III. VARIATIONS PRODUCED IN THE GLYCOGEN, LACTIC ACID AND PHOSPHORUS OF THE MUSCLE

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Carbohydrate metabolism in normal striated muscles involves many substances in complex interrelationships. Present views of these physiologic functions are presented by Meyerhof,<sup>1</sup> Hill,<sup>2</sup> Fiske and Subbarow<sup>3</sup> and Parnas.<sup>4</sup>

However, the changes of metabolism in pathologic conditions of muscle in the body have been investigated but little. We are concerned in this paper with acute molecular degeneration of striated muscle, especially as to its effect on certain factors of carbohydrate metabolism.

The work was carried out on rabbits, in the muscles of which acute molecular degeneration had been induced by a standard, easily controlled method previously described by us.<sup>5</sup> In addition, several rabbits were used for the study of variations in the glycogen and lactic acid of the muscles as produced in the lesser injury of freezing. In these animals, the muscles were frozen solidly with carbon dioxide snow, allowed to thaw spontaneously at room temperature and removed forty-eight hours later. The animals were young, healthy stock on an ordinary mixed diet. Both the injured and the control animals were made to fast for forty-eight hours before removal of the muscles, but were given water freely. They were kept in individual small cages that restricted activity.

Degenerated muscles were removed in the acute, florid stage of the process, from twenty-four to forty-eight hours after injury. With the

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From the Department of Pathology, Northwestern University Medical School.

1. Meyerhof, O.: *Chemical Dynamics of Life Phenomena*, Philadelphia, J. B. Lippincott Company, 1924.

2. Hill, A. V.: *Physiol. Rev.* **2**:310, 1922.

3. Fiske, C. H., and Subbarow, Y.: *J. Biol. Chem.* **81**:629, 1929.

4. Parnas, J. K.: *Compt. rend. Soc. de biol.* **101**:37, 1929.

5. Fishback, D. K., and Fishback, H. R.: *Am. J. Path.* **8**:211, 1932.

animal anesthetized by iso-amyl-ethyl barbituric acid, the muscle was removed after having been frozen *in situ* by a slush of carbon dioxide snow and ethyl chloride, according to the method of Davenport and Davenport.<sup>6</sup> It was then finely sliced while frozen hard and transferred to the required fluids for further chemical procedures, as described later.

Glycogen, lactic acid and phosphorus compounds were determined, since they represent important factors in carbohydrate metabolism in muscle. These will be considered separately in the following sections of this report.

#### GLYCOGEN

*Methods.*—About 1 Gm. of the finely sliced carbon dioxide-frozen muscle was weighed carefully and rapidly into ice-cold 60 per cent potassium hydroxide, and the mixture was shaken thoroughly. The quantitative determinations, of glycogen were made by the method of Pflüger,<sup>7</sup> with determinations of sugar on the final hydrolyzed samples by Somogyi's modification<sup>8</sup> of the Shaffer-Hartmann procedure.<sup>9</sup>

*Results.*—As represented in chart 1, the glycogen values obtained for the control animals vary considerably, but fall within a comparatively high range, with a maximum of 726, a minimum of 450 and an average of 567 mg. per hundred grams. Similar variability in normal muscle was reported by Handovsky and Westphal,<sup>10</sup> and by Davenport, Davenport and Ranson.<sup>11</sup> The high value, 953 mg., shown in the control group, was obtained on a rabbit that had not previously been made to fast and was not used in calculating the group average. It shows the effect of recent ingestion of food on the storage of glycogen in muscle.

With injury produced by previous freezing of the muscle there was a definite decrease of the glycogen store to a range of from 175 to 367 mg. per hundred grams. Mechanical trauma reduced the glycogen level still more, to the very low average of 104 mg. per hundred grams, the lowest value being 60 mg. Microscopic study of the degenerated muscles demonstrated structural changes of much greater severity in the mechanically injured muscles than in the frozen ones.

In the muscle injury studied here, the major blood vessels were found to be intact, although there was some capillary injury.<sup>5</sup> Thus

6. Davenport, H. A., and Davenport, H. K.: J. Biol. Chem. **76**:651, 1928.

7. Pflüger, E.: Arch. f. d. ges. Physiol. **129**:362, 1909.

8. Somogyi, M.: J. Biol. Chem. **70**:599, 1926.

9. Shaffer, P. A., and Hartmann, A. F.: J. Biol. Chem. **45**:365, 1921.

10. Handovsky, H., and Westphal, K.: Arch. f. d. ges. Physiol. **220**:399, 1928.

11. Davenport, H. A.; Davenport, H. K., and Ranson, S. W.: J. Biol. Chem. **82**:499, 1929.

the circulation was ample to nourish and keep the tissue alive. This type of experiment on muscle, with the general body relationships of the latter undisturbed, avoids the objections to metabolic experiments on extirpated muscles, in which systemic regulatory factors have been eliminated.

The injured muscle was not used by the animal, but the effect on the glycogen store of keeping the muscle at rest was negligible. Daven-

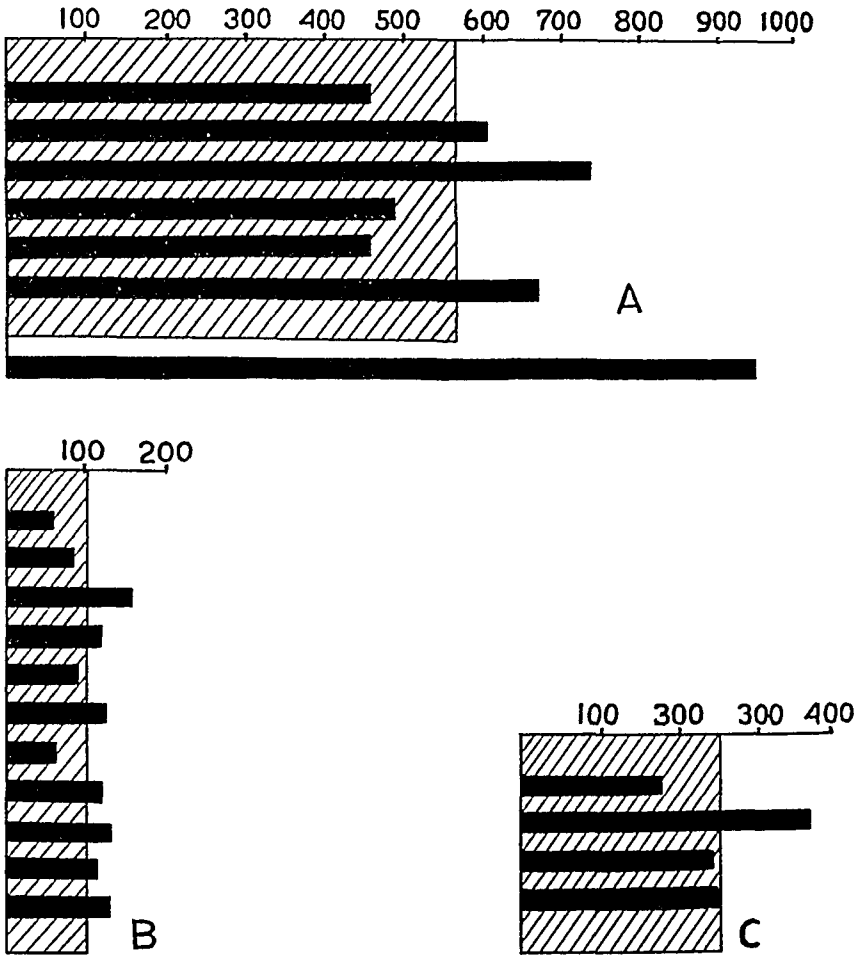


Chart 1.—Glycogen content (milligrams per hundred grams) of (A) control muscles, (B) contused muscles and (C) frozen muscles. The solid black rectangles represent individual values; the rectangles of diagonal lines, the group averages.

port, Davenport and Ranson,<sup>11</sup> working on muscle contracture, found no lowering of glycogen reserve from lack of contractile activity.

Many general systemic occurrences may alter the glycogen store of the muscles. Among these are general fatigue, fasting and hyperpyrexia, with lowering of the sugar supply. The same effect is obtained by administering thyroid, insulin or strychnine. Postmortem change in the muscles is associated with rapid loss of glycogen. A decrease of glycogen in the muscles is found with diabetes.

Experimentally, local fatigue of the muscle has been found to decrease the glycogen store.<sup>12</sup> Davenport, Davenport and Ranson,<sup>11</sup> working with experimental tetanic contracture of the hind legs of animals, found varied amounts of glycogen in different stages of the contracture in innervated muscles, but no alteration of the amounts of glycogen in denervated muscles. Denervation likewise protects the muscle against loss of glycogen, according to Wertheimer,<sup>13</sup> even in fasting, which causes general lowering of the level of muscle glycogen otherwise.

In this acute molecular degeneration of muscle there is found in every instance a rapid primary glycogenolysis, with a resultant low value on quantitative examination. The maintenance of glycogen at this low level for forty-eight hours or more with an adequate supply of sugar offered in the blood indicates probable depression of the glycogenic function, or of the glycogen-storing capacity of the injured muscle, in addition to the early glycogenolysis.

#### LACTIC ACID

*Methods.*—The finely sliced frozen muscle was extracted for ten minutes in ice-cold 5 per cent trichloroacetic acid. Lactic acid was determined on the filtrate by the method of Friedemann, Cotonio and Shaffer,<sup>14</sup> with Davenport's modification<sup>6</sup> for small amounts of tissue.<sup>15</sup>

*Results.*—The results are shown graphically in chart 2. In the control animals, the lactic acid content of the muscles averaged 17.3 mg. per hundred grams. In some of the earlier work, in which iso-amyl-ethyl barbituric acid alone was used to produce anesthesia, values ranged to almost 22 mg. Later, intraspinal injection of procaine hydrochloride was added, with the result that relaxation of muscles was complete, and the lactic acid values were lowered to around 15 mg. In the degenerated muscle, however, this factor was not significant, since the animal kept the injured parts motionless.

In the degenerated muscles there was marked increase of lactic acid. In those injured by freezing, the lactic acid reached an average value of 48 mg. per hundred grams, or an increase of 175 per cent over the average control value, and in those injured by mechanical trauma, it reached 64.6 mg., or an increase of 275 per cent.

Lactic acid is present in muscles normally as an intermediate product of sugar catabolism. According to Meyerhof,<sup>16</sup> the reaction dextrose→

12. Olmsted, J. M. D., and Coulthard, H. S.: *Am. J. Physiol.* **84**:610, 1928.

13. Wertheimer, E.: *Arch. f. d. ges. Physiol.* **215**:779, 1927.

14. Friedemann, T. E.; Cotonio, M., and Shaffer, P. A.: *J. Biol. Chem.* **73**:335, 1927.

15. Dr. Davenport loaned us his apparatus and gave valuable suggestions.

16. Meyerhof, O.: *Lancet* **2**:1415, 1930.

lactic acid is reversed during the stage of recovery of normal contractility, with reformation of part of the sugar.

Many procedures, such as mild exercise or slight interference with venous drainage from the muscle, cause increased formation and accumulation of lactic acid in the normal muscle. In muscles removed from the body, lactic acid accumulates rapidly. The rate of this formation of lactic acid is increased by trauma, such as cutting or grinding the muscle. In hashed muscle or extracts of muscle there is active

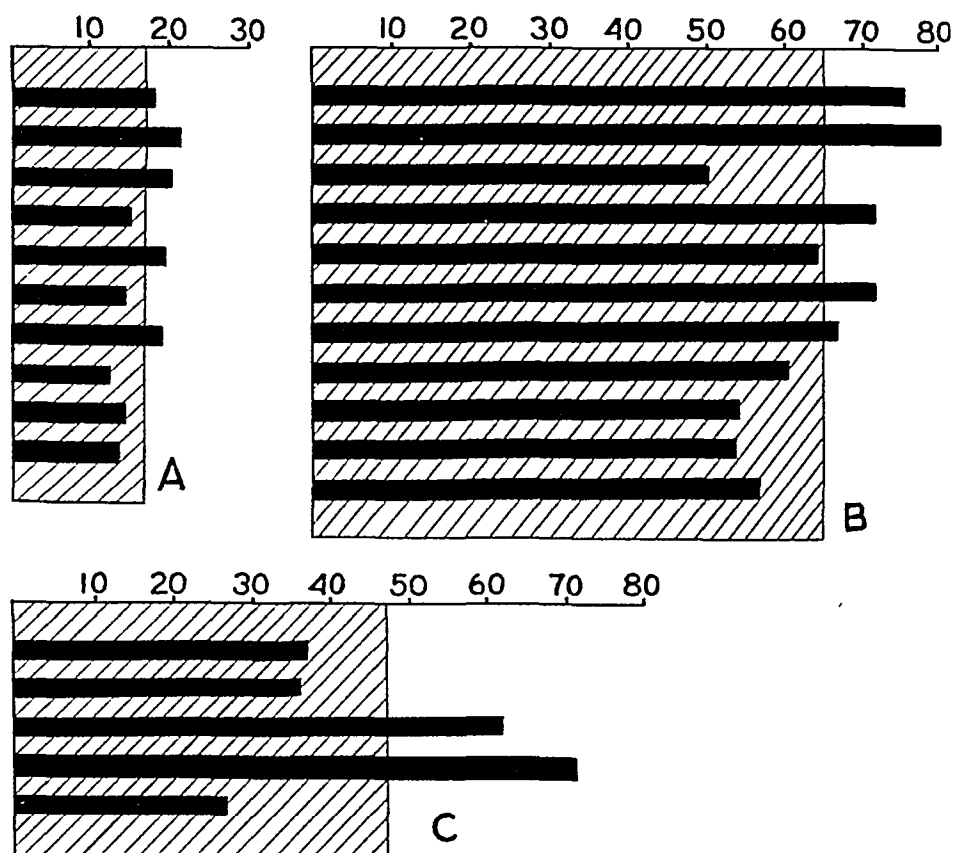


Chart 2.—Lactic acid content (milligrams per hundred grams) of (A) control, (B) contused and (C) frozen muscles. The solid black rectangles represent individual values; the rectangles of diagonal lines, the group averages.

glycolytic capacity, and on incubation of such digests large quantities of lactic acid may be found.<sup>17</sup> In an untraumatized muscle *in vitro*, such as a gastrocnemius of the frog, the rate of formation of lactic acid may be accelerated by stimulating the muscle to contraction. Abnormal accumulation of lactic acid may occur in the muscles of the living

17. (a) Embden, G.; Kalberlah, W., and Engel, H.: *Biochem. Ztschr.* **45**:45, 1912. (b) Meyerhof, O.: *ibid.* **183**:176, 1927. (c) Davenport, H. A., and Cotonio, M.: *J. Biol. Chem.* **73**:463, 1927. (d) Stiven, D.: *Biochem. J.* **23**:583, 1929.

animal with extreme fatigue<sup>18</sup> or with stasis of venous blood.<sup>19</sup> After death there is likewise an abnormal accumulation of lactic acid in the muscles, which is, according to Meigs,<sup>20</sup> an important factor in the causation of muscle rigor.

An active rôle is ascribed to lactic acid by Wells<sup>21</sup> in the production of Zenker's degeneration of muscle. In this study, we have applied the name "acute molecular degeneration of muscle," as previously suggested,<sup>5</sup> to cover the degenerative changes of muscle which include "waxy degeneration" as one phase. Such degeneration is undoubtedly constantly associated with increase in lactic acid, but the order of increase is not as high as may be found, in fatigue or post mortem, for instance, without degeneration of muscle cells.

The accumulation of acid in these injured muscles in amounts maintained well beyond normal values indicates damage to the metabolic function, with a resulting incomplete cycle of carbohydrate change. The process would doubtless reach an equilibrium at a far different level of substrate and products, as in digests of muscle hash, except for the variable factors introduced by the circulating blood.

#### PHOSPHORUS COMPOUNDS

*Methods.*—Phosphocreatine and inorganic phosphorus were determined by the method of Fiske and Subbarow<sup>3</sup> on the trichloroacetic acid extract of the muscle. (See section on lactic acid.) The addition of the carbon dioxide-frozen muscle to the cold trichloroacetic acid lowered its temperature below the freezing point of water, so that ice formed on the outside of the extraction flask. The ice-cold extract was filtered quickly into an iced receptacle containing enough saturated alkali to slightly alkalinize the filtrate, in order to minimize the hydrolysis of phosphocreatine.

*Results.*—In chart 3, the values shown for phosphocreatine in the control animals range within rather narrow limits, from 61 to 72 mg. per hundred grams, with an average of 68 mg. The inorganic phosphorus values of 30 to 37 mg. likewise show but little variation from the average of 34 mg.

In the degenerated muscles there is a marked drop of phosphocreatine to an average of 7 mg. per hundred grams. In carrying out determinations of the very low content of the injured muscles, a known amount of phosphate standard was added to the sample after the hydrolysis of the phosphocreatine, according to the suggestion of Fiske

18. Meyerhof, O., and Lohmann, K.: *Arch. f. d. ges. Physiol.* **204**:327, 1924.

19. Mendel, B.: Engel, W., and Goldscheider, I.: *Klin. Wchnschr.* **4**:307, 1925.

20. Meigs, E. B.: *Am. J. Physiol.* **26**:191, 1910.

21. Wells, H. G.: *J. Exper. Med.* **11**:1, 1909.

and Subbarow. Inorganic phosphorus values of the injured muscles showed only a slight inconstant increase over control values, with an average of 37 and a range of from 32 to 45 mg. per hundred grams.

The low phosphocreatine is the most marked feature in the altered phosphorus metabolism of the injured muscles. Similar levels have been reported also for muscles that were stimulated to contraction to the state of fatigue.<sup>22</sup> With time allowed for recovery, and with blood circulation intact, the phosphocreatine of fatigued muscles rather rapidly

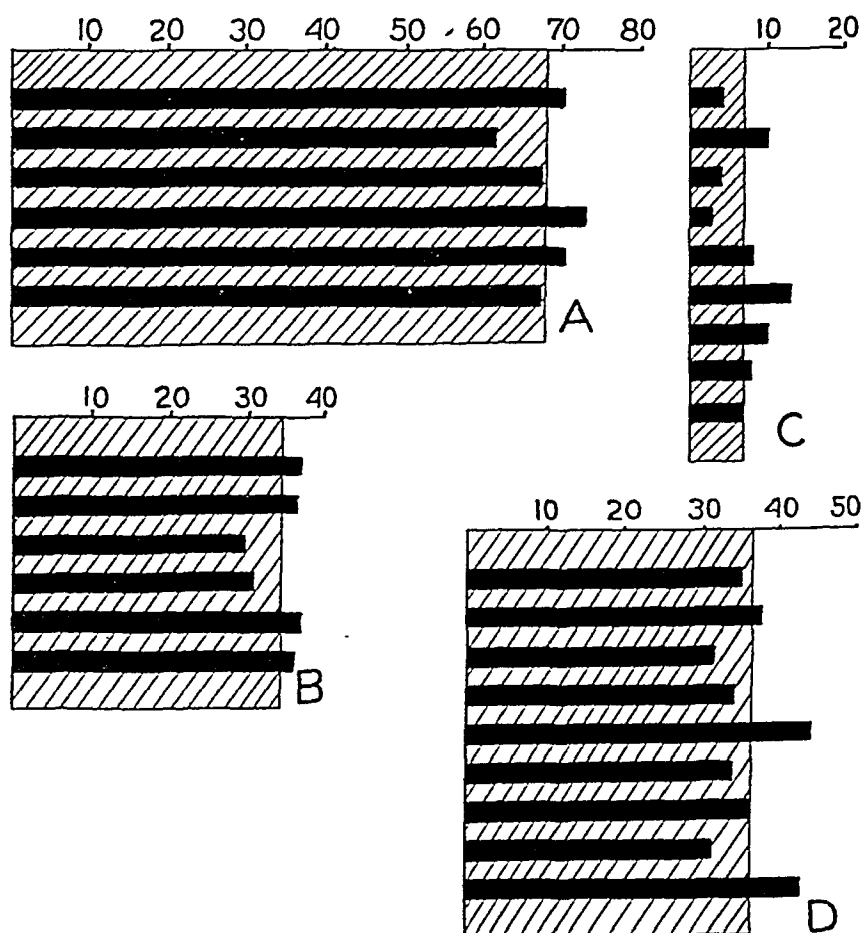


Chart 3.—(A) Organic and (B) inorganic phosphorus content of control muscles (milligrams per hundred grams); (C) organic and (D) inorganic phosphorus content of degenerated muscles. The solid black rectangles represent individual values; the rectangles of diagonal lines, the group averages.

recovers. In fatigue, then, the low values may be explained as the driving of the reaction in the direction of hydrolysis of phosphocreatine with insufficient time for the reverse process, or recovery, to occur.<sup>22</sup>

22. (a) Irving, L., and Bastedo, G. M.: *Am. J. Physiol.* **86**:225, 1928.  
 (b) Dixon, H. H.; Davenport, H. A., and Ranson, S. W.: *J. Biol. Chem.* **82**:61, 1929. (c) Fiske and Subbarow.<sup>3</sup>

23. Meyerhof, O., and Nachmansohn, D.: *Biochem. Ztschr.* **222**:1, 1930.

The factor of time is not significant, however, in explanation of the low values in injuries that are 48 hours old, far longer than the short period of recovery in uninjured fatigued muscles. Since there is also an ample supply of blood to these injured muscles,<sup>5</sup> the cause of the impaired phosphocreatine balance must be in the altered metabolism of the pathologic muscle cells themselves.

Inorganic phosphorus amounts remain close to the control values. This is quite different from the results reported for incubated muscle hash, muscle extract or expressed juice of muscle,<sup>24</sup> in which the decrease in phosphocreatine is associated with a corresponding increase in inorganic phosphorus. Probably the latter values represent the end-result or equilibrium in a closed system, which would not be likely to be reached in the body, with the number of variable factors introduced by the circulating blood.

Under anaerobic conditions in muscle, according to Meyerhof,<sup>16</sup> phosphagen hydrolyzes without increase in inorganic phosphorus, the new-formed phosphoric acid being combined with hexose. In these injured muscles, even with sufficient supply of blood, it is still possible that within the individual units of degenerated muscle fibers anaerobic conditions may have prevailed.

It does not seem likely that any significant increase in inorganic phosphorus has been lost by diffusion out of the injured muscles in the given period. Further, so far as loss by circulation is concerned, it was stated by Irving and Bastedo<sup>22a</sup> that even though twice the blood content of inorganic phosphorus was formed in a muscle, no increase in inorganic phosphorus could be found in the venous drainage.

#### COMMENT

In this acute injury of muscle the degenerative lesion is diffuse and extensive. Metabolic function might be expected to be disordered in keeping with the morphologic changes, when such changes are found to involve the parenchymatous cells.

The marked decrease in glycogen, the rise of lactic acid, and the fall of phosphocreatine are evidence of disturbance of the cycle of reactions in sugar metabolism.

The trend of the reaction is the same as in digests of muscle hash or muscle extract. It might be inferred that the variation from the normal metabolism in acute molecular degeneration of muscle is brought about by enzyme activity which is freed from the control usually exerted by the living cell over these intracellular functions.

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24. Beattie, F., and Milroy, T. H.: *J. Physiol.* **60**:379, 1925; **62**:174, 1926. Embden, G., and Haymann, C.: *Ztschr. f. physiol. Chem.* **137**:154, 1924. Meyerhof,<sup>17b</sup> Davenport.<sup>17c</sup>



Since the normal muscle metabolism plays an important rôle in the maintenance of body heat, extensive injury of muscle in disease, with a lowering of the easily combustible carbohydrate store of the muscles, may well be a serious factor in the body economy. The acute molecular degeneration occurring with typhoid fever may thus be a factor in the pronounced asthenia and subnormal temperature of the convalescence. The same may be true in other severe acute infections <sup>25</sup> in which acute molecular degeneration of muscle has been found. In this connection, pure speculation would suggest still further that variable degrees of disturbance of muscle cell metabolism may exist in the body in disease, with morphologic changes not demonstrated by the present crude technic.

#### SUMMARY

A uniform acute molecular degeneration of muscles produced in rabbits has been found to be associated with alteration of the muscle metabolites.

Muscle glycogen dropped consistently from a control value of 567 mg. per hundred grams to an injury average of 104 mg. per hundred grams.

There was a marked rise of lactic acid from the control average of 17.3 mg. to 64.6 mg. per hundred grams.

Phosphocreatine decreased from the control average of 68 mg. to 7 mg. per hundred grams. Inorganic phosphorus varied slightly and irregularly from the control value, with a slight tendency toward increase.

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25. Stenström, B.: *Arch. Path.* **3**:361, 1927. Stemmler, W.: *Virchows Arch. f. path. Anat.* **216**:57, 1914.

# Laboratory Methods and Technical Notes

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## A SIMPLE METHOD FOR THE STUDY OF THE SPORULATION OF COCCIDIAL OOCYSTS

K. WAGENER, V.M.D., LINCOLN, NEB.

The sporulation of coccidial oocysts is one of the most important criteria of exogenous development of parasites, and at the same time is of importance in the identification of the different genera of coccidia. Therefore, this test is extensively applied in laboratory practice. The methods of the test must include consideration of the optimum conditions of sporulation of the oocysts. At present these conditions are known sufficiently, as far as general points of view are concerned, but much less in their details.

Generally a sufficiently high temperature, sufficient humidity and the presence of oxygen are regarded as absolutely necessary for abundant and quick sporulation.

The more common methods of testing the sporulation of coccidial oocysts aim to correspond to these conditions as far as possible. However, they show some differences. The method recommended by Nöller<sup>1</sup> of spreading the feces to be tested on some layers of humid filter paper in a Petri dish allows undisturbed exposure to oxygen, and by the cover of the Petri dish desiccation is prevented or delayed. Kept at room temperature, the oocysts, with this method, show abundant sporulation, mostly within two or three days. In another method widely used for testing the sporulation of oocysts, as mentioned by Pérard,<sup>2</sup> Tyzzer,<sup>3</sup> Henry,<sup>4</sup> Becker and Crouch<sup>5</sup> and others, the feces to be tested are suspended in a 2.5 per cent solution of potassium dichromate, which prevents decomposition, on the one hand, and acts as an oxidizing reagent on the other. Also by this method the oocysts usually develop spores within two or three days.

In my investigations, in the course of which both these methods were used, the following manner of observing the sporulation proved to be more satisfactory and useful, especially in serial experiments in which the interest centered on the microscopic examination.

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From the Department of Animal Pathology and Hygiene, University of Nebraska.

1. Nöller, W., in Stang and Wirth: *Tierheilkunde und Tierzucht*, Berlin, Urban & Schwarzenburg, 1928.

2. Pérard, C.: *Compt. rend. Acad. d. sc.* **179**:1436, 1924.

3. Tyzzer, E. E.: *Am. J. Hyg.* **10**:269, 1929.

4. Henry D. P.: *Univ. California Publ., Zool.* **36**:157, 1931.

5. Becker, E. R., and Crouch, H. B.: *Proc. Soc. Exper. Biol. & Med.* **28**:529, 1931.

## METHOD

The feces containing the oocysts are spread thinly on the surface of a plain agar solution, poured and solidified in Petri dishes. The agar used for this purpose is a simple solution in distilled water commonly used for bacteriologic mediums. In order that the correct degree of humidity of the medium may be secured, the amount of agar to be dissolved in distilled water has to correspond to its degree of solidification, which varies a little in the different commercial brands of agar. In my investigations, in which Bacto-agar (Difco standardized) was used, an amount of from 0.8 to 1 per cent proved to be sufficient. This agar is moist enough to favor sporulation, but also solid enough to allow an easy spreading of the feces on its surface. The humidity that gathers on the surface of the solid agar and on the cover of the Petri dish keeps the medium and the atmosphere moist during the period of examination. Addition of preservatives or of disinfectants has not been necessary.

At room temperature, the sporulation usually takes place within from twenty-four to forty-eight hours, as in the case of the other two methods described.

The advantage of the agar plate for testing sporulation in diagnostic and systematic serial examinations is that the preparation of smears as otherwise necessary for microscopic examination thus becomes superfluous. The Petri dishes are simply put on the stage of the microscope, after the removal of the cover, and examined, first, by low power magnification. More often the sporulation can be seen with the low power magnification, but there is no objection to use of the higher magnification also, after a cover glass has been put on the portion of the plate surface to be examined. After cedar oil has been placed on the cover glass, the immersion lens can also be used. This method saves much time in larger series of examinations. Care has to be taken only that the plates when poured shall be as thin as possible, as thicker layers are apt to show vibrations, which make examination more difficult. By using this method, the influence of some disinfectants on oocysts can be ascertained by adding the disinfectant in a proper concentration to the agar solution.

The eggs of some internal parasites, which were occasionally added to the medium with the feces containing oocysts, showed segmentation and embryonization, when they were kept long enough under observation. Therefore, the plain agar medium might also be used for the study of the eggs of parasites; for such more extended examinations, it might be recommended that the surface of the medium be moistened with some drops of water from time to time.

## SUMMARY

From 0.8 to 1 per cent of agar dissolved in distilled water and poured into Petri dishes constitutes a simple medium for studying the sporulation of coccidial oocysts. The feces containing the oocysts are smeared thinly on the surface of the agar and can be examined directly under the microscope by using the low magnification, the high magnification, or even the oil emersion systems.

# General Review

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## THROMBOSIS AND FATAL PULMONARY EMBOLISM

COMPARISON OF THEIR FREQUENCY IN THE CLINICS OF  
CENTRAL EUROPE AND NORTH AMERICA, WITH  
SPECIAL REFERENCE TO INCREASE

SOL ROY ROSENTHAL, M.D.

CHICAGO

In discussing the problems of the increase of thrombosis and pulmonary embolism, one necessarily considers the statistical reports. Such data are at best open to criticism especially as different types of clinical material are reported and as different pathologists have varying interest in these conditions. To avoid as much error as possible, the statistics of each pathologist must be considered as a definite entity. Only the interpretations may be compared and not the actual numerical values.

Hegler, in 1926, was first to call attention to the marked rise of thrombosis in St. Georg Hospital, Hamburg, and since that time there have been many reports relative to this question. The majority of the reports have come from the clinics of Central Europe. Other countries, including the United States, have few, if any, published reports on the subject.

In order to make this review as complete as possible, a questionnaire was sent to leading pathologists in this country. The answers cover representative sections.

Because the reports in this country appear to be different from the European, many of the concepts relative to the pathogenesis of thrombosis and embolism are open to question.

The most direct approach to the interpretation of the reports from the various clinics was found in plotting their values in the form of graphs. Only the percentages were plotted, but these showed such wide variations that a scale had to be used in which corresponding results could be telescoped onto one chart and in which the smaller

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From the Department of Pathology, Cook County Hospital; Dr. R. H. Jaffé, Director.

values were not completely overshadowed by the larger ones. The solution of this scale was found in the semilogarithmic four cycle paper, suggested to me by Dr. A. Bachem, biophysicist, at the University of Illinois, College of Medicine. The four cycles are in progression of ten. The smallest value was placed at 0.01, making the largest value 100. From these charts direct values may be read, it being remembered that the coordinate is in a logarithmic scale and represents the percentage of thrombosis or of embolism, while the abscissa is in a linear scale and represents the year.

By maintaining a common scale, the angle of inclination or decline in any given instance can be compared; and by drawing a straight line through each curve, determined by approximately bisecting the values, the mean angle of inclination is obtained. This angle designates the trend of increase or of decrease in thrombosis or in embolism over the years reported. In the charts, the individual curves are represented by an interrupted line, while the average angle of inclination is represented by a continuous line, except where otherwise indicated.

As not all reports lent themselves to plotting in graph form, tables have been constructed to show the percentages of thrombosis and fatal pulmonary embolism of the earliest and latest years embraced in the study. Occasionally other years are mentioned that might influence the report. The reports are derived entirely from postmortem material.

THE INCIDENCE OF THROMBOSIS IN GENERAL CLINICS OF  
CENTRAL EUROPE (TABLE 1; CHARTS 1 AND 7A)

The scope of most of the reports covers the years from 1910 to 1930. From the line representing the average angle of inclination, a universal elevation of thrombosis is evident. The lowest increase as deduced from this line is from  $4\frac{1}{2}$  to  $9\frac{1}{2}$  per cent (Cologne), while the highest increase is from 12 to 30 per cent (Leipzig). To elucidate these interpretations, the following example is cited. In the report of Adolph and Hopman from the Augusta Hospital in Cologne, the percentage of thrombosis in 1912 was 7.5 and in 1927, 9; an increase of 1.5 per cent. When 1926 is considered with a percentage of 10.5 and 1924 with a percentage of 4, the greatest increase is  $6\frac{1}{2}$  per cent. The average increase as deduced from the line of inclination is 5 per cent.

That an increase has taken place is claimed universally by the authors reporting, but in some instances there is a slight tendency to return to normal. This can be studied best in chart 7A, where is indicated, year by year, the number of clinics denoting tendencies to increase or decrease in thrombosis. In scanning this chart it must be borne in mind that the general trend is toward increase, and that chart 7A was constructed from chart 1. Each year is viewed by itself, and the inclination of the respective curves is indicated.

As the World War is considered the most important factor about which the increase in thrombosis and embolism revolves, the study of the corresponding graph is divided into three periods—before the war, from 1910 to 1914; during the war, from 1914 to 1918, and after the war, from 1918 to 1930.

Before the war, as many clinics showed increase as decrease. In 1914 there was a sudden rise in four clinics with a decrease in two.

TABLE 1.—*The Incidence of Thrombosis and Embolism in the General Clinics of Central Europe*

City and Author	Thrombosis		Fatal Pulmonary Embolism	
	Year	Percentage	Year	Percentage
Hamburg: Wertheimer.....	1910	1.48	1910	0.44
	1929	11.0	1929	2.9
Düsseldorf: Schleussing.....	1911	10.7	1911	0.4
	1928	17.0	1928	1.8
Göttingen: Grüber.....	....	....	1911	0.68
			1927	3.46
Budapest: Bodin.....	....	....	1911-1913	0.3
			1918-1928	1.12
Leipzig: Singer and Morawitz.....	1912	15.6	1912	1.5
	1928	29.7	1928	6.9
Stettin: Auxhausen.....	1912	6.9	1912	3.4
	1928	14.0	1928	8.8
Cologne: Adolph and Hopman.....	1912	7.5	1912	1.8
			1919	0.8
	1926	10.5	1926	3.4
	1927	9.0	1927	1.9
Munich: Oberndorfer.....	1912-1914	5.0	1912-1914	2.0
	1925-1927	12.0	1925-1927	5.0
Hamburg: Hegler.....	1913	5.0	1926	50 emboli
	1925	10.0	1927	82 emboli
Wiesbaden: Schulz.....	1915-1920	2.1	1915-1920	1.0
	1928 (1st half)	12.0	1928	11.3
Freiburg: Kuhn.....	1919	18.6	1919	1.61
	1927	24.5	1927	4.94
Rostock: Höring.....	....	....	1922	1.9
			1927	6.8
Thrombosis and Embolism				
Munich: Martini and Oppitz.....	1924	16.4		
	1927	23.6		

During 1915, 1916 and 1917, there was again a remission, as there were as many reports of decrease as of increase. In 1919 and 1920, the tendency was toward increase, and in the years from 1921 to 1928 it was so without exception. In 1929, a decline set in, but the level was still high, much higher than in the prewar period.

One can deduce, then (charts 1 and 7 A), that the actual ascent of the incidence of thrombosis began in 1919, became universal in 1922 and reached its height in 1928. The most pronounced increase was from 1921 to 1927. In 1929, a slight trend for a return to normal is indicated.

THE INCIDENCE OF FATAL PULMONARY EMBOLISM IN GENERAL  
CLINICS OF CENTRAL EUROPE (TABLE 1;  
CHARTS 2 AND 7 B)

Similar to thrombosis, fatal pulmonary embolism has shown a definite increase in the general clinics of Central Europe. The lowest average values were from 1.6 to 2 per cent (Cologne), while the highest values were from 0.44 to 4 per cent (Hamburg, Wertheimer).<sup>1</sup> This increase is not generally as striking as that in thrombosis. The average percentage of increase in thrombosis surpasses that in fatal pulmonary embolism.

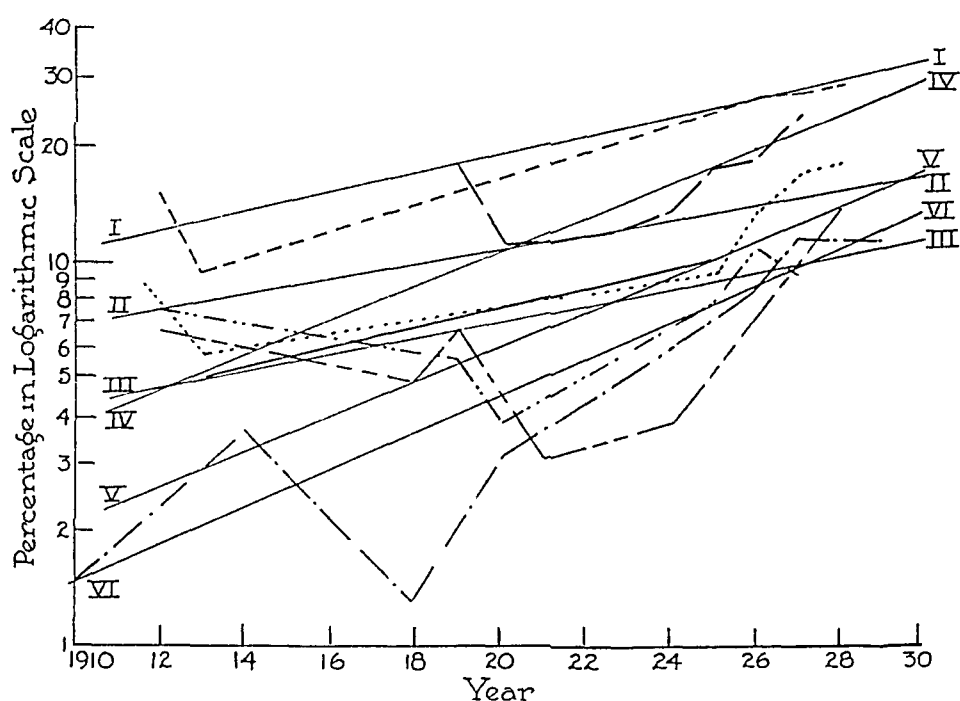


Chart 1.—The incidence of thrombosis in the general clinics of Central Europe: I. Leipzig; II. Düsseldorf; III. Cologne; IV. Freiburg; V. Stettin; VI. (dash and dot); University, Hamburg; VII. (solid line), St. Georg Hospital, Hamburg.

Some authors claim a tendency to return to the prewar number of fatal pulmonary embolisms (Adolph and Hopman, Cologne; Bodin, Budapest).

In considering yearly the inclination of fatal pulmonary embolism (charts 2 and 7 B), it appears that before the war there was a decline, while during the war and one year after it there was an increase. In 1920, the increase was definitely established, although not in all cases. In 1923, a decline was found in 36 per cent of the clinics reporting, but the level was above that of the prewar period. Another height was

1. The increase of fatal pulmonary embolism in Wiesbaden was from 1 per cent to 11.3 per cent, but these values did not lend themselves to plotting and are given only in table 1.

reached in 1925; then followed a slight remission to a higher level than in 1923, and finally the highest percentages were reached in the years from 1926 to 1928. After this, many of the clinics (50 per cent) began to show a decline or a stationary level.

The relationship between thrombosis and fatal pulmonary embolism was directly proportional until 1922, when the percentage of thrombosis

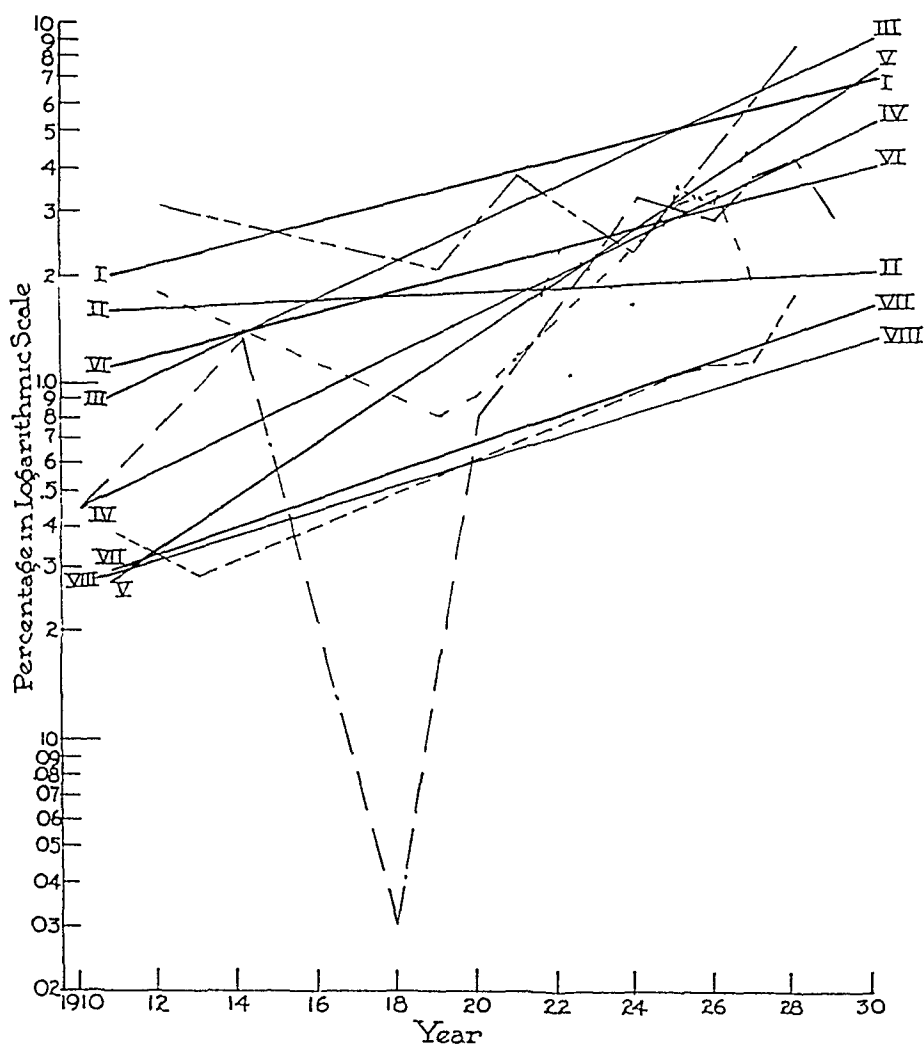


Chart 2—The incidence of fatal pulmonary embolism in the general clinics of Central Europe I. Stettin; II. Cologne; III. (no curve), Leipzig; IV. Hamburg; V. Freiburg; VI (no curve), Gottingen; VII, Dusseldorf; VIII (no curve), Budapest

continued to rise while that of embolism fell. This reversal continued until 1923, when the percentage of embolism rose and continued to do so up to 1925. From this time on, some of the clinics began to show a decline, although the majority still demonstrated an increase. Thrombosis during this entire period was steadily climbing. The acuity of inclination for embolism was also less marked than that for thrombosis.



In summary, the incidence of fatal pulmonary embolism in the general clinics of Central Europe has been on the increase. The rise began with the war, but did not definitely assert itself until 1920. Except for a slight remission, the elevation continued until 1928. The highest percentage was reached during the years from 1926 to 1928, after which 50 per cent of the clinics showed a tendency toward constancy or decrease. The increase in thrombosis was more prolonged than that in pulmonary embolism. The average increase in thrombosis was more pronounced than that in embolism.

TABLE 2.—*The Incidence of Thrombosis and Embolism in the Surgical Clinics of Central Europe*

City and Author	Thrombosis		Fatal Pulmonary Embolism	
	Year	Percentage	Year	Percentage
Hamburg: Hegler.....	1913	0.06		
	1925	0.4		
Vienna: Stöhr and Kazda.....	....	....	1915	0.4
			1925	1.6
Heidelberg: Sulger and Boszin.....	....	....	1919	2.9
			1926	17.4
			1928	12.3
Göttingen: Geissendorfer.....	....	....	1919	0.17
			1924	0.36
			1928	0.18
Freiburg: Killian.....	....	....	1919	0.09
			1930	0.38
Leipzig: Sarafoff.....	1920	0.33	1920	0.45
	1929	1.07	1929	5.1
Würzburg: Bauer.....	1923	0.27	1923	0.2
	1928	1.15	1928	0.41
Leipzig: Sellheim (gynecology).....	1916	1.8	1914	0
	1928	2.5	1928	28.0
Leipzig: Sellheim (obstetrics).....	1914	0.05	1914	0
	1928	0.7	1927	0.15
Tübingen: Mayer (obstetrics).....	1914	1.9	1914	0.17
	1929	2.0	1929	0.5

THE INCIDENCE OF FATAL PULMONARY EMBOLISM IN THE  
SURGICAL CLINICS OF CENTRAL EUROPE (TABLE 2;  
CHARTS 3 AND 7C)

An increase in fatal pulmonary embolism has been observed generally in the surgical clinics of Central Europe. Although the majority of the reports begin with 1919, the authors stated that up to that time the percentage was uniformly low. The average range of increase varied from 0.2 to 0.32 per cent (Göttingen) and from 2 to 16 per cent (Heidelberg). The upper and lower limits are similar to those found in the general clinics, but the average angle of inclination is higher in the surgical clinics.

Judging from the reports, the incidence of fatal pulmonary embolism was rather uniform until 1921. In 1922, a definite rise set in, which with one exception became generalized. But for a slight remission in

1925, the rise continued, reaching its high peaks in 1926 and 1927. In 1928, the tendency was toward a decrease or a standstill, but the level, except in one instance, was higher than that during the first years reported.

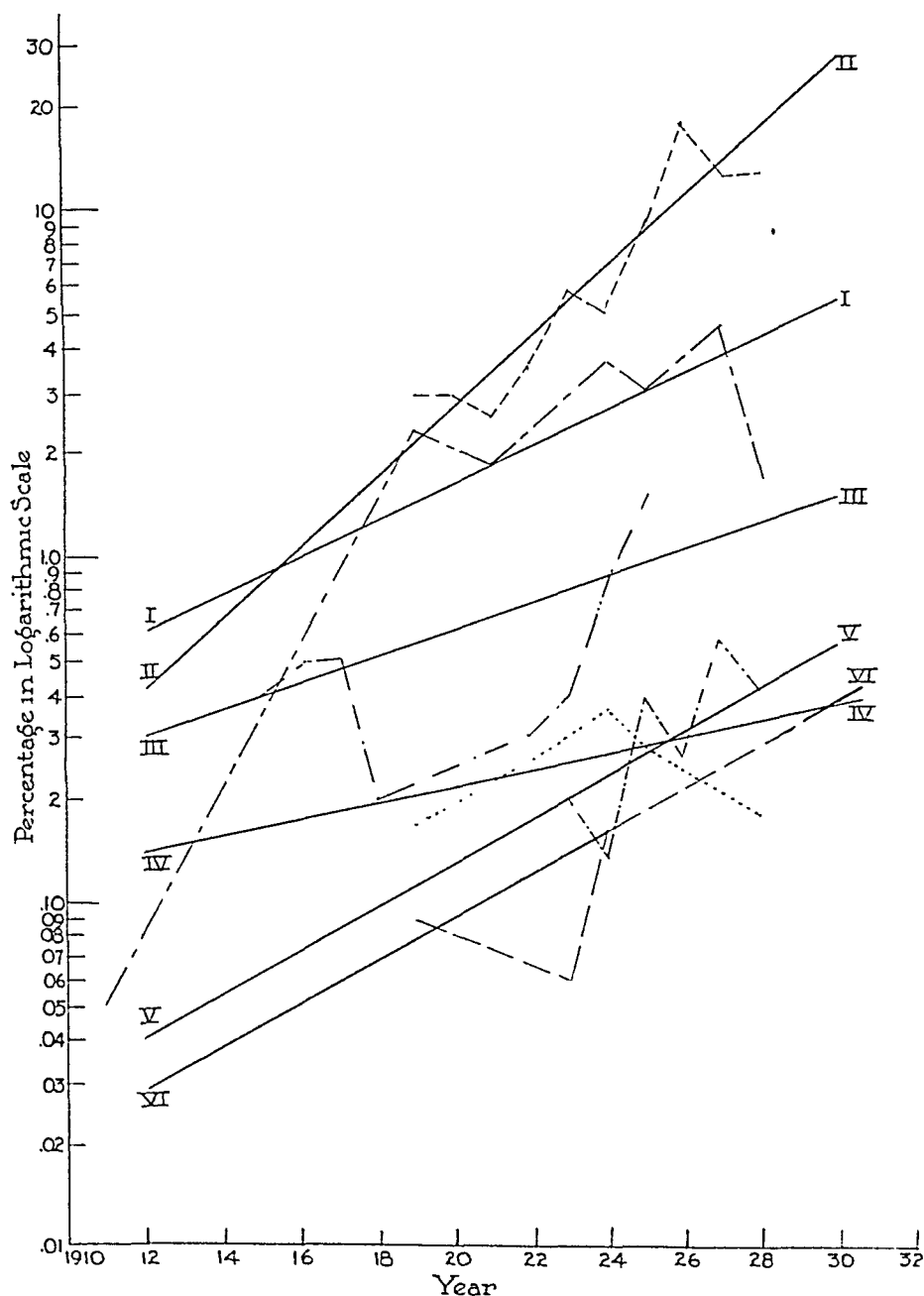


Chart 3.—The incidence of fatal pulmonary embolism in the surgical clinics of Central Europe: I, Budapest; II, Heidelberg; III, Vienna; IV, Göttingen; V, Würzburg; VI, Freiburg.

In summary, the increase in fatal pulmonary embolism in the surgical clinics of Central Europe was pronounced and much more so than in the general clinics. The rise began in 1922 and reached its peak in

1926 and 1927. In 1928, a decline set in, but the level was higher than that given in most previous reports.

Data on thrombosis in the surgical clinics of Central Europe are limited. The four curves cited in chart 4 all show an upward tilt. No comparisons are made.

The purely gynecologic and obstetric clinics (table 2) are few. However, many of the general and surgical clinics mention the increase in thrombosis and embolism in gynecologic and obstetric clinics (Sellheim, Leipzig; Mayer, Tübingen; Kuhn, Freiberg; Bodin, Budapest).

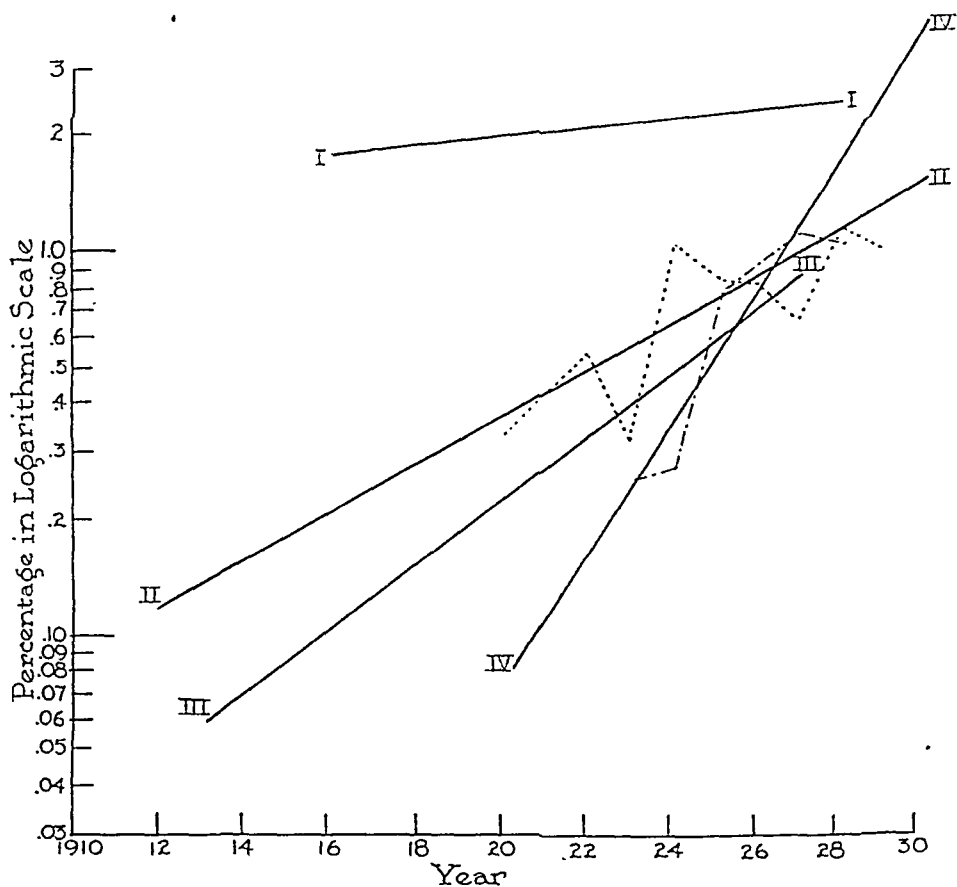


Chart 4.—The incidence of thrombosis in the surgical clinics of Central Europe: I, Leipzig (gynecologic clinic); II, Leipzig; III, Hamburg (St. Georg Hospital); IV, Würzburg.

The gynecologic clinics showed a more pronounced increase of fatal pulmonary embolism than the obstetric clinics.

#### THE STATISTICS ON THROMBOSIS AND ON FATAL PULMONARY EMBOLISM IN THE CLINICS OF NORTH AMERICA

These statistics were obtained, save in the case of the Mayo Clinic, by a questionnaire sent to the directors of the pathologic institutes all over the country. An obvious rise of thrombosis and embolism is not manifest.

The data came mainly from general clinics. However, on thrombosis there are two, and on fatal pulmonary embolism three, reports from surgical clinics. Only postmortem material was considered.

THE INCIDENCE OF THROMBOSIS IN THE CLINICS OF NORTH AMERICA (TABLE 3; CHART 5)

These reports are necessarily considered in groups, according to the span of years covered in each case. Of the five reports dating back

TABLE 3.—*The Incidence of Thrombosis and Embolism in the Clinics of North America*

Clinic and Author	Thrombosis		Fatal Pulmonary Embolism	
	Year	Percentage	Year	Percentage
Presbyterian Hospital, New York: Pappenheimer-Robinson	1913-1914 1930-1931	25.7 20.9	1913-1914 1930-1931	11.1 6.2
University of Minnesota: McCartney	....	....	1913 1929 1930	1.0 0.7 1.7
Stanford University Medical School: Ophüls and Dobson	1913 1931	6.7 23.0	1913 1931	1.0 1.5
Northwestern University, affiliated hospitals: Simonds and Dyrenforth	1914 1916 1920 1930	10.0 28.0 14.0 20.8	1918 1930	1.7 1.5
Los Angeles General Hospital: Evans	1917 1929 1931	1.5 4.5 6.7	1917 1926 1930 1931	0 0.2 0.1 0.3
Toronto General Hospital: Koltz, Belt and Smith	1926 1930	18.0 16.4	1926-1930	3.1
Cincinnati General Hospital: Austin	1926 1930 1931 (1st half)	0.23 2.1 4.2	1920 1930 1931	2.1 1.0 2.4
Cook County Hospital, Chicago: Jaffé, Rosenthal	1929 1930 1931	13.4 10.7 6.0	1929 1930 1931	0.2 0 0.33
Presbyterian Hospital, surgical clinic, New York: Palmer	1913 1928 1930	0 0.77 1.27	1913 1928 1930	0.05 0.66 0.4
Stanford University Medical School, surgical clinic: Ophüls and Dobson	1913 1931	1.0 0.2	1913 1917 1931	0 1.5 0.5
Mayo Clinic, surgical dept., Rochester, Minn.: Hendersen, Walters	....	....	1917-1927 1926-1930	0.34 0.09

to the prewar period (table 3), that for the general clinic of the Presbyterian Hospital in New York and that for the surgical clinic of Stanford University Medical School show a decrease, while three show an increase: Northwestern University hospital affiliates, the surgical clinic of Stanford University Medical School and the surgical clinic of the Presbyterian Hospital, New York. The Los Angeles General Hospital, reporting from 1917, shows an increase. Of the three clinics reporting from 1926 on, the Cincinnati General Hospital shows an increase and the Toronto General Hospital and the Cook County Hospital, Chicago, a decrease.

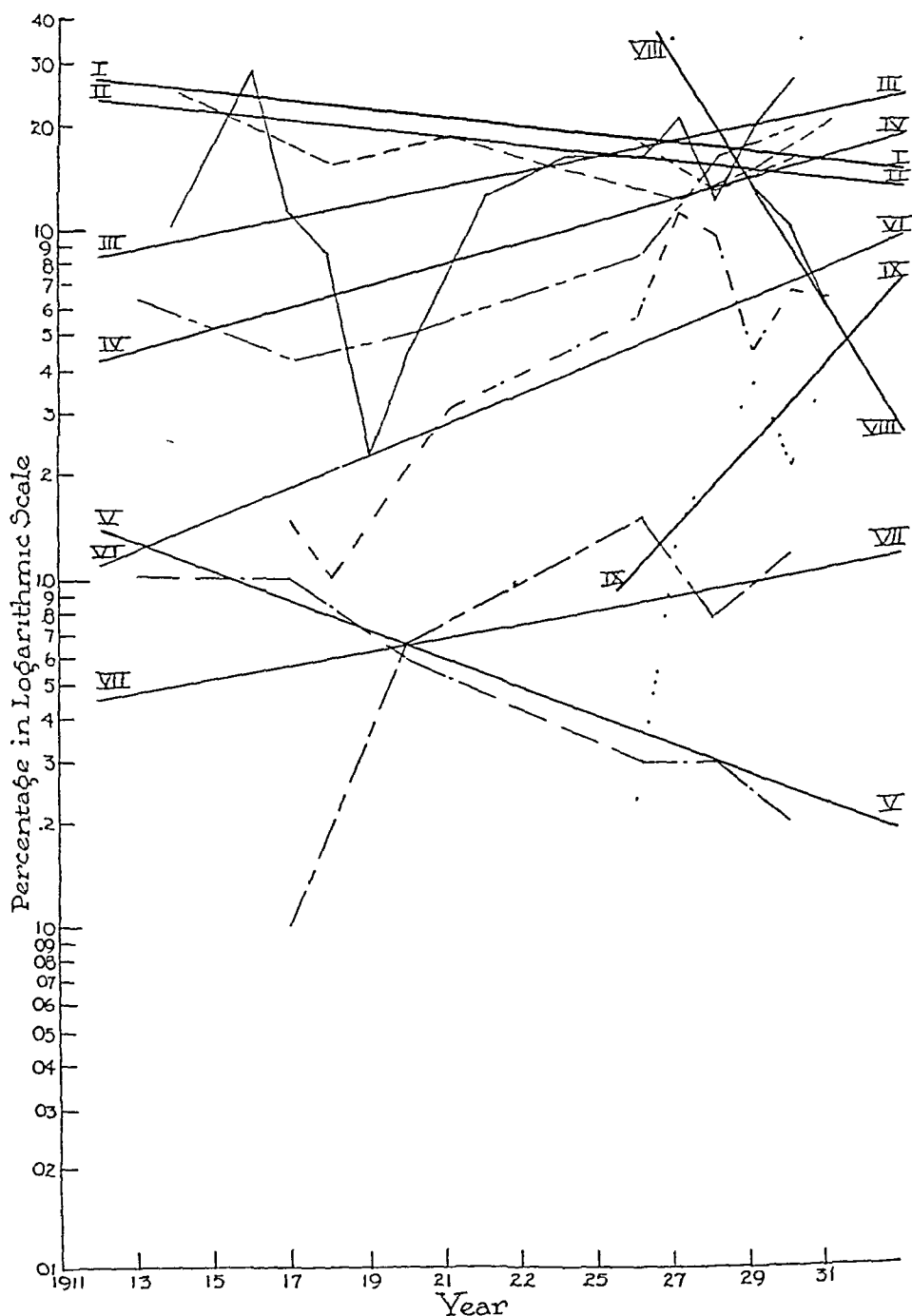


Chart 5.—The incidence of thrombosis in the clinics of North America: I, Toronto General Hospital; II, Presbyterian Hospital, general clinic, New York; III, Northwestern University and affiliated hospitals, Chicago; IV, Stanford University, medical clinic; V, Stanford University, surgical clinic; VI, Los Angeles General Hospital; VII, Presbyterian Hospital, surgical clinic, New York; VIII, Cook County Hospital; IX, Cincinnati General Hospital.

Taking these reports together, one may deduce that concerning the period between 1914 and 1931, opinion has been divided as to increase in thrombosis. It is impossible thus to arrive at any definite conclusions. Because the statistics for fatal pulmonary embolism are more complete, and because, generally speaking, the incidence of thrombosis and that of embolism run parallel, further deductions will be offered on pulmonary embolism.

THE INCIDENCE OF FATAL PULMONARY EMBOLISM IN THE  
CLINICS OF NORTH AMERICA (TABLE 3; CHART 6)

These reports lend themselves more appropriately for comparison with those of Central Europe. Their scope in the majority of instances embraces the prewar period (1913 to 1931) and, with the exception of one instance, the results show a decrease, no change or a slight increase.

Specifically, the clinic showing an increase is the surgical clinic in the Presbyterian Hospital, New York, from 0.09 to 0.8 per cent; but to counteract this rise, the other surgical clinics showed a fall, from 0.34 to 0.09 per cent, Mayo Clinic, and from 1.5 to 0.5 per cent, Stanford University Medical School. The remaining eight reports give no indication of an increase in deaths from pulmonary embolism, but indicate rather a decrease.

In summary, there has been no increase in fatal pulmonary embolism in the clinics of North America. Because of the usual parallelism between thrombosis and embolism, and because the opinions as to the incidence of thrombosis are divided and incomplete, one may deduce that there has been no increase in thrombosis.

In comparing the reports of the Central European and the North American clinics, caution is exercised because of the discrepancy in the number of clinics reporting and in the number of years included in the reports. However, it must be borne in mind that the European statistics have been instigated by the alarm caused by an apparent increase in thrombosis and pulmonary embolism. All of these reports have been published, while the North American statistics are the result of a questionnaire sent out by me. In other words, there has been no noticeable rise in thrombosis and embolism in this country to warrant publication. To bear this out, the North American reports, when complete, show a tendency toward decrease rather than increase. This is especially true in regard to pulmonary embolism, about which this paper is most concerned.

In view of these circumstances, many factors held responsible for the pathogenesis of thrombosis and embolism may be excluded.

The European authors point at old age, cardiac and vascular changes, modern methods of therapy (especially intravenous), postwar inflation (1919 to 1924), surgical procedures (including anesthetics), nutrition,

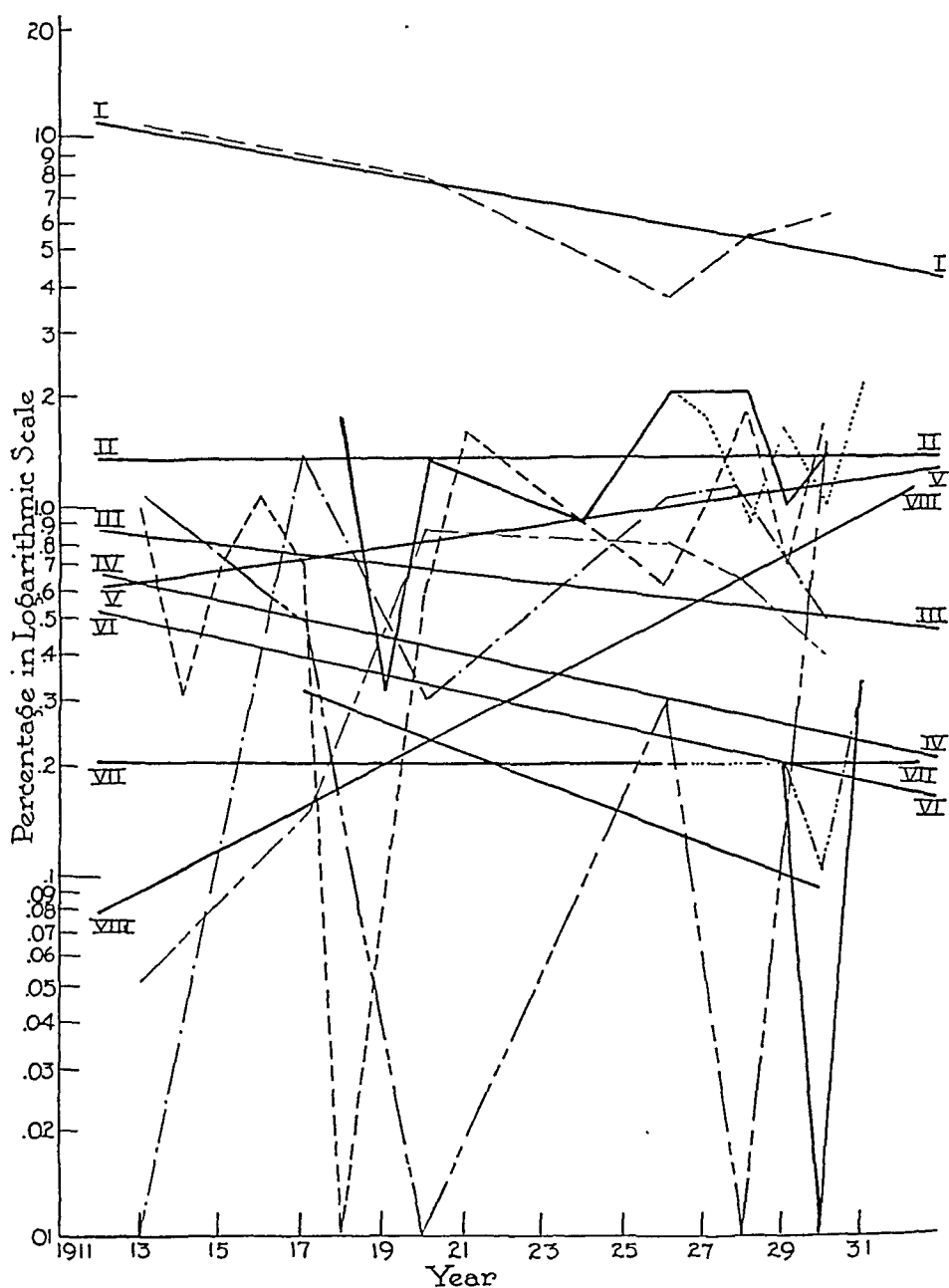


Chart 6.—The incidence of fatal pulmonary embolism in the clinics of North America: I, Presbyterian Hospital, general clinic, New York; II, Northwestern University and affiliated hospitals, Chicago; III, Stanford University, surgical clinic; IV, Stanford University, medical clinic; V, University of Minnesota; VI, Cook County Hospital; VII, Los Angeles General Hospital; VIII (long dash-short dash), Presbyterian Hospital, surgical clinic, New York; VIII (solid line), Mayo Clinic, Rochester, Minn.

the influenza epidemic and changes of the weather, as influencing the rise of thrombosis and pulmonary embolism. These conditions will be considered later and compared with the conditions in North America, especially at the Cook County Hospital, Chicago, in which 2,500 consecutive autopsies have been reviewed.

#### THE RELATIONSHIP OF AGE TO THE RISE OF THROMBOSIS AND PULMONARY EMBOLISM

Without exception, the European authors have found the greatest number and percentage of cases of thrombosis and fatal pulmonary embolism in persons between the ages of 40 and 70 years (Kuhn, Klinke, Singer and Morawitz, Oberndorfer, Höring, Adolph and Hopman, Sellheim, Geissendorfer, Killian, Grüber, Hutter and Urban, Schulz, Bauer, Qure). The greatest number of cases occurred in persons between the ages of 40 and 50 years, while the highest percentage was that of cases occurring in persons between 60 and 70 years of age.

In this country, the average age for thrombosis and pulmonary embolism, as found in the Toronto General Hospital, Canada, in the Northwestern University affiliated hospitals, Chicago, and in the Stanford University Medical School, San Francisco, was about 50 years. This does not include the age incidence with the highest percentage. At the Cook County Hospital, Chicago, the greatest number of cases occurred in persons between 41 and 50 years of age, while the highest percentage was that of cases in persons between 61 and 70 years of age.

In summary, the age incidence for thrombosis and fatal pulmonary embolism in Central Europe is similar to that in this country (where reports are available). That this factor alone is responsible for an increase, thus becomes doubtful.

#### THE RELATIONSHIP OF CARDIAC AND VASCULAR CHANGES TO THE RISE OF THROMBOSIS AND PULMONARY EMBOLISM

Observations directed to the circulatory system reveal that changes in the heart and, to a lesser extent, in the blood vessels are in the majority of instances associated with thrombosis and embolism. The Central European reports designate this relationship in from 51.7 per cent (Axhausen) to 95 per cent (Bauer) of the cases. It is of interest to note that not only general clinics proclaim this as a factor of utmost importance (Höring, Fahr, Oberndorfer, Martini and Oppitz, Kahn, Klinke, Singer and Morawitz, Schleussing, Schulz, Bodin, Adolph and Hopman, Axhausen, Wertheimer) but also surgical clinics (Nippe, Sellheim, Sarafoff and Bauer). Bauer pointed out that although only 20 per cent of the patients who died of pulmonary embolism at the Würz-



burg surgical clinic presented circulatory disturbances clinically, 95 per cent of them showed myocardial and vascular changes on postmortem examination.

Thrombosis and pulmonary embolism were associated with cardiac and vascular lesions in over 80 per cent of the cases at the Cook County Hospital and in 52 per cent of the cases at the Stanford University Medical School.

In summary, cardiac and vascular conditions play an important rôle in thrombosis and embolism, both in Central Europe and in this country (Hendersen, Walters, Polak, Rosenthal, Ophüls and Dobson).

#### THE RELATIONSHIP OF SURGICAL PROCEDURES TO THE RISE OF THROMBOSIS AND EMBOLISM

Although some authors report a greater increase of thrombosis and embolism in the medical clinics (Axhausen, Kuhn, Klinke, Schleussing, in 1929), the majority of authors state that the rise has been more pronounced in the surgical clinics (Geissendorfer, Bodin, Schleussing [1924-1927], Höring, Bauer, Hueck, Wahlig, Sulger and Boszin, Killian, Hegler, Stöhr and Kazda). This can best be seen by comparing charts 2 and 3

Of the surgical procedures, laparotomy is more frequently associated with thrombosis and embolism (Geissendorfer, Bodin, Höring, Bauer, Hueck, Kuhn, Detering, Patey, Petren, Ducuing, Miller and Rogers, and McCartney). The usual order of frequency of thrombosis and embolism in relation to the organs is as follows: prostate, stomach, gallbladder, appendix, hernia (Höring, Geissendorfer, Bauer). It is well to remember in this regard that the associated lesions in prostatic hypertrophy are, as a rule, hypertrophy of the heart with dilatation, and urinary infection.

Because of the frequency of thrombosis and embolism following surgical procedures, the nature of the blood, preoperative and postoperative, has been studied in the search for an explanation. The results of these researches will be discussed later. It is sufficient to mention here that changes in the bleeding and clotting time, in the concentration and sedimentation and in carbon dioxide, lipid and calcium content have all been given as factors influencing the formation of thrombi. Yet these factors do not account for the increase in thrombosis, as surgery is practiced in this country on a huge scale, while the incidence of thrombosis and fatal lung embolism has not increased.

In summary, the greatest increase in thrombosis and pulmonary embolism in the Central European clinics was found to follow surgical procedures. But neither this fact nor postoperative changes in the

blood explain the increased incidence, for surgery is practiced in this country to about the same extent as in Central Europe.

#### THE RELATIONSHIP OF MEDICATION TO THE RISE OF THROMBOSIS AND EMBOLISM

The modern methods of intravenous therapy have been considered to play an important rôle in the high incidence of thrombosis and embolism (von Linhard, Oehler, Fahr, Hegler, Reye, Martini and Oppitz, Kuhn). These authors agree with Martini and Oppitz that although a large percentage follows intravenous injections (38 per cent) a higher percentage follows intravenous therapy in cases of heart disease (81 per cent).

On the contrary there are as many reports showing that intravenous therapy has played but a slight rôle in the increase of thrombosis (Adolph and Hopman, Bodin, Schulz, Wahlig, Schleussing, Oberndorfer, Singer and Morawitz, Hueck, Höring). Some of these authors have gone so far as to say that the greatest increase in thrombosis and embolism has occurred in patients who did not receive intravenous medication (Adolph and Hopman, Hueck). Oberndorfer and Höring have stated that the modern intensive cardiac therapy has been a greater factor in the increase in thrombosis than intravenous injection.

It cannot be denied that intravenous medication of intensive cardiac therapy applied to older patients with cardiac lesions predisposes to the formation of thrombi. But the factor of therapy alone cannot be held responsible for the rise in thrombosis and embolism, as these newer methods of medication are used extensively in this country and yet no increase in thrombosis and embolism has been noted.

There are occasional reports of pulmonary embolism following intravenous injections of bismuth (Garcia, Joulia, Petges and Joulia), arsenic (Burn and Bromberg) and corrosive solutions used to occlude varicose veins (Silverman). These instances are rare. Silverman found for corrosive solutions an incidence of less than 0.015 per cent. This does not consider the thousands of clinics using this form of therapy, in which no pulmonary embolism has occurred.

In summary, intravenous injections and intensive cardiac therapy per se have not been responsible for the increase in thrombosis and embolism.

#### THE RELATIONSHIP OF NUTRITION TO THE RISE OF THROMBOSIS AND PULMONARY EMBOLISM

Because fatal pulmonary embolism was found more frequently in well nourished to obese persons, it was deduced that the improvement of nutrition in the postwar inflation period might bear a direct relation-

ship to it (Deider, Kuhn, Schulz, Wahlig, Bauer). That pulmonary embolism frequently occurs in obese persons has been noted also in this country (Hendersen, Snell, Polak and Mazzola, Rosenthal). But in a previous report, I have shown that, in the well nourished, 84 per cent of the cases of thrombosis and embolism were associated with cardiac and vascular changes as compared with 64 per cent in the undernourished. Of the four fatal cases of pulmonary embolism in 2,500 autopsies at the Cook County Hospital, all were in obese women, with hypertrophic and dilated hearts.

The augmentation of cardiac and vascular diseases is thus of greater importance in the formation of thrombi and emboli than the betterment of nutrition. The foods of the Central European peoples during the inflation period, high in carbohydrate and low in vitamins, must have had much bearing on the increase in heart disease, for, as has been shown by Ohmori, a deficiency of vitamin B leads to dilation of the heart.

In summary, pulmonary embolism is most common in obese persons, but the obesity is probably of less significance than the cardiac and vascular changes.

#### THE RELATIONSHIP OF THE INFLUENZA EPIDEMIC TO THE RISE OF THROMBOSIS AND EMBOLISM

The epidemic of influenza that followed the war is considered by some (Franke) to have influenced the increase in thrombosis and embolism. Franke stated that the infection directly affected the endothelium and led to thrombus formation. Bodin and Kuhn could find no relationship between the epidemic and the increase in thrombosis and embolism. Cajigal reported only one case of death from pulmonary embolism in 100 postmortem examinations during the influenza epidemic. This may be taken to mean that there was not an increase in pulmonary embolism in Barcelona during 1919. A similar observation was made by Nagajo of Tokyo.

In the United States, the epidemic was widespread and intense. Yet there has been no increase in thrombosis and embolism.

In summary, the influenza epidemic played no rôle in the increase of thrombosis and embolism.

#### THE RELATIONSHIP OF WEATHER CONDITIONS TO THE RISE OF THROMBOSIS AND EMBOLISM

Opposite views are held regarding the effect of weather conditions on thrombosis and embolism. Killian of Freiburg, in analyzing the weather charts of that locality, pointed out that during July and August, the dry south winds favored the formation of thrombi because

of the increased perspiration and evaporation. He found most cases of thrombosis and embolism in October, July and August and the fewest in June and September. The recent increase of southerly winds, Killian felt, partly influenced the rise of thrombosis and embolism.

On the contrary, Fritzsche, Klinke, Geissendorfer, Bauer and Sarafoff showed that the most cases of thrombosis occurred during the wet, stormy and cold weather. Bauer explained this by the fact that

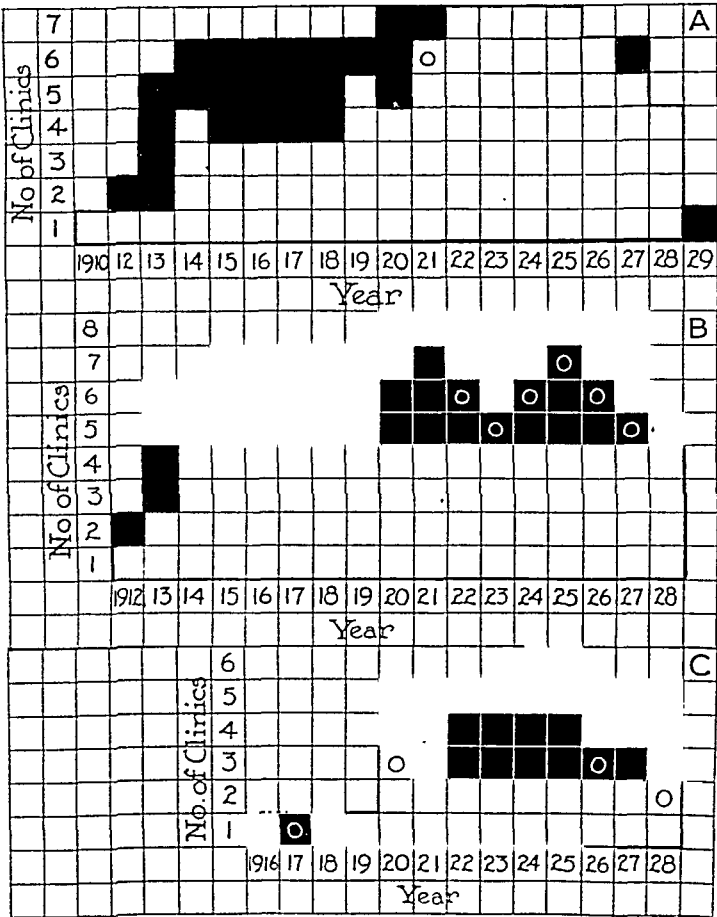


Chart 7.—The relationship between the increase and decrease of (A) thrombosis in the general clinics, (B) fatal pulmonary embolism in the general clinics and (C) fatal pulmonary embolism in the surgical clinics, of Central Europe. The white square indicates increase; the solid black square, decrease, and a circle, no change.

there are twice as many illnesses in the winter as in the summer. Fritzsche's stand was that during wet and stormy weather, the increase of electric charge of the air acts on the autonomic nervous system and influences the vascular mechanism. This promotes stasis and leads to thrombosis. The same author also stated that the present mode of living, especially in the early postwar period, resulted in instability of the autonomic nervous system.

The reports from this country are derived from all quarters, moist, dry, hot and cold, but there has been no increase in thrombosis and embolism. As to the present mode of living, in this country, it probably surpasses in eccentricity and momentum that of Central Europe.

In summary, weather conditions and changes in the mode of living do not account for the elevation of thrombosis and embolism.

#### COMMENT

In attempting to explain the rise in thrombosis and embolism in the Central European clinics, the pathogenesis of these conditions has been studied, and modern methods of chemical and physical analysis have been applied. Although these studies have added much to the present conception of thrombosis, the problem is far from being solved. The earlier theories of John Hunter (1794), who spoke of vascular changes as a result of infection, of Andral (1842), who added the factor of stasis and finally of Virchow (1885), who believed that changes in the blood, vascular alterations and stasis, whether associated or not associated with infection, may lead to thrombosis, are still in the foreground.

Stasis alone, from an experimental standpoint, does not terminate in thrombus formation. This was first shown by Baumgarten in 1877, and has been verified by Miller and Rogers, and by Armentrout. On adding endothelial injury to stasis, Baumgarten and more recently Armentrout (1931) reported thrombus formation. It is questionable whether true thrombi were produced. More likely, mere coagulation took place, for Miller and Rogers, using sixty-three cats, were unable to produce true thrombi by double ligation of veins with scarring of the endothelium, interposition of muscle, etc. They did produce one thrombus at a site where infection had set in. Similar results were obtained by Dietrich and Schröder.

Thus, experimentally, stasis and endothelial injury do not lead to thrombosis, although both may play important rôles. It follows, then, that blood changes are prerequisite, and, indeed, all modern writers agree in that regard. As to the nature of these changes, there is much disagreement. The increased incidence of surgical procedures has been offered as influencing the rise of thrombosis and embolism. Extensive studies of the blood have established increased blood protein through absorption from surgical wounds (Zschau, Stöhr and Kazda); increased blood calcium, carbon dioxide content, sedimentation and concentration (Qure); increased fibrinogen and globulin, which rob the red blood corpuscles and platelets of their electrical charge and promote agglutination (von Seeman, Stuber and Lang); shortening of the clotting and bleeding time (Sulger, Boszin); colloidal chemical and physical changes in the blood (Höring, Allen, Wildegans), and increase in

blood platelets from the eighth to the eleventh day postoperatively, when the majority of thrombi are formed (Hueck, Miller).

Undoubtedly many of these factors may influence thrombosis, but they are not necessarily related to operative procedures. After injections of sugar solutions or in carcinoma, an increase in the globulin and fibrinogen content of the blood has been demonstrated (Stuber and Lang, Wildegans); an increase in platelets and blood protein has been found following a diet high in protein (Milles); clotting time is decreased in infection (Jürgens); carbon dioxide content of the blood is increased in infections, which leads to a decrease in the platelet charge (Prima); calcium and cholesterol are increased in atherosclerosis (Gechtman and Slauskey). Finally, surgical procedures have also increased to a great extent in this country, with no corresponding increase in thrombosis and embolism.

Thus one may conclude that the changes in the blood observed postoperatively may not be the result of surgical procedures, and that the increase in surgical operations does not account for the increase in thrombosis and embolism.

Dietrich championed the importance of infection in thrombosis. He agreed with Klemensiewicz that a thin layer of a homogeneous substance forms on the endothelium at the site of the thrombus. This, he explained, is the result of a sensitization of the endothelium, after which there is a direct reaction between endothelium and blood to form the thrombus. All these factors are directly influenced by chronic infections; stasis merely acts in localizing the clot. Experimentally, sensitization of an organism has resulted in foci of endothelial proliferation. Actual thrombosis has been produced when suppuration was introduced with stasis (Dietrich and Schröder, Miller and Rogers). Jürgens, by the use of a capillary thrombometer, found a decrease in clotting time in infections.

Suppuration and infection, then, alter the blood and in some instances also the endothelium. The most important rôle of the latter condition is, however, its effect on the blood generally, for in the material at the Cook County Hospital, it was found that generalized infections and remote suppurations by far outnumbered proximal suppurations in relation to thrombosis.

Agreeing that infections and suppurations play a part in thrombus formation, what bearing has that on the increase in thrombosis and embolism? With the first report of an increase in the latter conditions, Hegler emphasized the concomitant increase in diseases generally—pernicious anemia, chlorosis, endocarditis, abscess of the lung, grippe and bronchopneumonia. An increase in the incidence of infectious processes has also been reported by Schleussing and Klinke.

In this country there have been no reports of any increase in infections or in thrombosis and embolism. It seems probable, then, that a higher incidence of infections and suppurations in Central Europe may account partly for the increase in thrombosis and embolism.

As has been stated, the European authors and a survey at the Cook County Hospital have shown that cardiac and vascular changes are frequently associated with thrombosis and embolism. Stasis in heart failure and endothelial injury alone do not account for thrombus formation. There must exist changes in the blood. These changes may result from increased destructive processes during the descending stage of life (40 years onward, Aschoff), increased carbon dioxide content of the blood in myocardial insufficiency (Friedemann) or increased calcium and cholesterol in atherosclerosis (Gechtman and Slauskayu).

That there has been an increase in cardiac and vascular diseases following the war is widely accepted in Central Europe; much more so than in infections and suppurations (Wertheimer, Sellheim, Höring, Oberndorfer, Fahr, Bauer, Klinke, Kuhn, Martini and Oppitz). The explanations offered for this increase are: weakened condition of the heart as a result of hunger and nervous irritability which became manifest long after the war (Wertheimer, Sellheim), changes in the autonomic nervous system that accelerated cardiac and vascular changes (Fritzsche) and changes in the vascular system and the myocardium from epidemics affecting these systems (diphtheria, 1915 to 1918, meningitis, 1924, Klinke).

Thrombosis and embolism began to increase in 1919; the increase advanced in 1922 and extended to the period from 1926 to 1928. These years correspond fairly well to the postwar inflation period that began in 1919. During this period, as stated, the diet of the people in Central Europe consisted mainly of carbohydrates with little vitamin-containing food. This was a result of abandonment of the strict distribution of food. The injurious effects of deficiency in vitamins are well known. The lack of vitamin B causes dilatation of the cardiac chambers (Ohmori). This lack, then, should be included among the factors responsible for the increase in cardiac and vascular disease.

The rise of circulatory disturbances and that of thrombosis and embolism run almost parallel in the clinics of Central Europe. It is the former factor, then, which may be largely responsible for the increase in thrombosis and embolism.

#### SUMMARY

A survey of the literature discloses an increase in the incidence of thrombosis and pulmonary embolism in the clinics of Central Europe.

This rise was prevalent in the general, as well as the surgical, clinics, although more marked in the latter.

As a rule, the actual ascent began in 1919, became universal in 1922 and reached its height in 1928. Later than 1928 a decline became manifest.

The increase in thrombosis was more prolonged than that in pulmonary embolism.

The cause of the increase in thrombosis and embolism was largely the rise of cardiac and vascular disease and to a less extent the increase in infections and suppurative diseases.

The accentuation of cardiac and vascular diseases is explained by the hunger, nervous irritability and lack of vitamins that were especially manifest during the period of inflation (1919 to 1924) in Central Europe.

Reports from clinics in the United States and Canada fail to show an elevation in the incidence of thrombosis and pulmonary embolism.

The conflict in the reports from this country and from Europe makes it necessary to question many of the concepts relative to the pathogenesis of thrombosis and embolism. The factors that can be discounted are: operative procedures, weather conditions, the influenza epidemic, intravenous therapy and extensive heart therapy.

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## Notes and News

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**University Notes, Promotions, Resignations, Appointments, Deaths, etc.**—Graham Lusk, professor of physiology at Cornell University Medical College and scientific director of the Russell Sage Institute of Pathology, died on July 19, 1932, at the age of 66.

Frank P. Underhill, professor of pharmacology and toxicology in the school of medicine of Yale University, died on June 28, 1932, at the age of 55. He was professor of pathologic chemistry in Yale University from 1913 to 1918.

Bernhard Bang, professor of pathology and therapy in the Royal Veterinary and Agricultural College in Copenhagen, Denmark, died on June 22, 1932, at 83 years of age. Educated in human medicine, he became a leader in veterinary medicine. He devised a method, known as Bang's method, for the eradication of bovine tuberculosis, and isolated the bacillus of infectious abortion (Bang's bacillus).

According to *Science*, members of the staff of the Rockefeller Institute for Medical Research have accepted appointments as follows: Phillip Levine, instructor of pathology and bacteriology in the University of Wisconsin; J. Lionel Alloway, assistant professor of bacteriology in Cornell University Medical College; Ralph Knutti, resident pathologist at the Strong Memorial Hospital, Rochester, N. Y., and Douglas Sprunt, assistant professor of pathology in the medical school of Duke University.

**Society News.**—The Ohio Society of Clinical and Laboratory Diagnosis has appointed Jonathan Forman, Columbus, O., as chairman of a committee to work with a committee from the Ohio Embalmers' Association in the interest of securing more autopsies under proper conditions throughout the state of Ohio.

The International Association for the Study of Parodontosis has been organized. The first annual meeting was held at the University of Zurich on Aug. 7, 1932. Dr. Hermann Becks, Hooper Foundation for Medical Research, Second and Parnassus avenues, San Francisco, is the American representative.

**Study of Whooping Cough.**—A grant of \$25,000 from the Rockefeller Foundation to finance a four-year study of whooping cough has been announced by Western Reserve University. Gerald S. Shibley, associate professor of medicine in the medical school, will conduct the investigation, which will cover etiology, prevention, diagnosis and treatment. Cases appearing in the dispensary of Lakeside Hospital and in the community will furnish the material for observation. James Angus Doull, professor of hygiene and public health, will cooperate in the study.

**Graduate Fortnight of the New York Academy of Medicine.**—The next Fortnight, October 17 to 28 inclusive, 1932, will be devoted to the discussion of tumors. At the same time an exhibit of anatomic specimens will be set up in the Academy building. Further information may be obtained from the Academy, 2 East 103d Street, New York.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

OSTEOGENESIS IMPERFECTA ASSOCIATED WITH LESIONS OF THE PARATHYROID GLANDS. T. C. WYATT and T. H. McEACHERN, *Am. J. Dis. Child.* **43**:403, 1932.

In the case described there were marked congenital dysplasia of bone and unusual vascularity of the parathyroid glands with a relatively small amount of parenchymal tissue. A brief review of osteogenesis imperfecta and of the relation of the parathyroid glands to disease of bone is given. The possibility is suggested that so-called osteogenesis imperfecta may sometimes be based, in part, on a congenital parathyroid disturbance rather than on faulty mesoblastic differentiation.

AUTHORS' SUMMARY.

EFFECT OF CABBAGE FEEDING ON THE MORPHOLOGY OF THE THYROID OF RABBITS. I. T. ZECKWER, *Am. J. Path.* **8**:235, 1932.

Feeding winter cabbage in the early part of 1931 to seventeen rabbits for periods up to one hundred and fourteen days produced hyperplasia of the thyroid gland, but only in two instances did it result in an enlargement more than twice the normal weight. The microscopic changes of hyperplasia were more conspicuous than the gross enlargement. Under the conditions of these experiments, the feeding seemed to favor a high incidence of intercurrent infections. The data, in so far as a small series of experiments permits conclusions, support the view that there is annual variation in the goiterogenic agent of cabbage.

AUTHOR'S SUMMARY.

THE DYNAMIC BRONCHIAL TREE. CHARLES C. MACKLIN, *Am. Rev. Tuberc.* **25**:393, 1932.

The lop-sided inspiratory enlargement of the pleural cavity makes it necessary for the lung, in inspiration, to shift itself so as to occupy the new space, and thus one finds the hilus, in this phase, moving downward, forward and outward, and, in expiration, reversely. This oscillation is marked in forcible breathing, and is necessary to the proper ventilation of the part of the lung lying above and behind the hilus. Much depends on the physical condition of the root, which normally is flexible, allowing this adaptational movement to take place freely. When, however, the root is stiffened from disease processes, which may originate in early life, the movement is hampered or abolished, and then the subapical region, in particular, suffers. Thus it is suggested that impairment of the flexibility of the root may have a good deal to do with the site of election of a tuberculous process. Diagrams are used to make clear the points brought out, and a plea is made for concerted attention of clinicians to the lung root, to the end that its physiology and pathology may be better understood.

C. C. MACKLIN.

EFFECTS OF CINCHOPHEN IN RATS. HERBERT S. REICHLE, *Arch. Int. Med.* **49**:215, 1932.

Subcutaneous administration of cinchophen in single doses of 1 Gm. per kilogram of body weight killed rats within twenty-four hours. Continued parenteral administration of smaller doses (0.2 Gm. per kilogram) did not cause death, but after the last two injections the rats displayed symptoms that may possibly be

regarded as expressions of hypersensitiveness. Feeding of cinchophen in doses of approximately 20 mg. a day to rats when the livers had been depleted of glycogen by starvation or injured by chloroform did not induce cirrhosis. On postmortem examination, no characteristic changes in the histologic appearance of the organs were found. A review of the histories of cases of reaction to cinchophen suggests that its cause may be found either in natural or in developed hypersensitiveness.

AUTHOR'S SUMMARY.

EXPERIMENTAL VENTRICULAR DISTORTION AND CONVULSIONS IN THE CAT.  
S. B. WORTIS, Arch. Neurol. & Psychiat. **27**:776, 1932.

Aseptic laceration of the brain without removal of the products of trauma, in the cat, results in ventricular distortion due to meningeocerebral adhesions and contracting cerebral cicatrix. Head trauma resulting in the escape of blood into the cerebrospinal fluid often gives rise to mild bilateral ventricular dilatation in the absence of grossly demonstrable meningeocerebral adhesions or a cerebral scar. Aseptic laceration of the brain and head trauma resulting in fracture of the skull increase the animal's sensitiveness to a standard convulsant over the period of observation.

AUTHOR'S SUMMARY.

TRAUMATIC SUBDURAL HEMATOMA. W. J. GARDNER, Arch. Neurol. & Psychiat. **27**:847, 1932.

In the subdural hematoma there occurs a gradual increase in size. This progression is due to environmental conditions, particularly to inadequacy of lymphatic drainage from the mesothelium-lined subdural space. The increase is due to an accession of tissue fluid, particularly spinal fluid, which is drawn into the hemorrhagic cyst through the semipermeable arachnoid membrane and the adjacent wall of the cyst by the osmotic tension of the blood proteins contained in the cyst. It is difficult, if not impossible, to reproduce in the dog the clinical picture of subdural hematoma.

AUTHOR'S SUMMARY.

THE MONRO-KELLIE HYPOTHESIS OF CONSTANT INTRACRANIAL CONTENT.  
L. H. WEED and L. B. FLEXNER, Bull. Johns Hopkins Hosp. **50**:196, 1932.

Two series of experiments on animals were carried out, in which records were kept of the pressure of the cerebrospinal fluid, of the sagittal venous pressure and of the carotid arterial pressure. In the first series, the cranial dura was widely exposed to the atmosphere, while in the second series the vertebral arches were removed throughout the thoracic and lumbar regions, thus exposing a large extent of the spinal cord directly to atmospheric pressure. In the animal with the cranial dura exposed, the alteration in the pressure of the cerebrospinal fluid, on abrupt tilting from the horizontal to the vertical tail-down position, was considerably less than the alteration of pressure in this fluid in an intact animal on such a tilt. When the animal with cranial dura exposed was tilted from the horizontal to the vertical head-down position, the alteration of pressure was of exactly the same magnitude as in the intact animal. In the series with the vertebral canal opened, the changes in pressure in the cerebrospinal fluid, on tilting from the horizontal to the vertical tail-down position, were of exactly the same magnitude as in the intact animal. On tilting from the horizontal to the vertical head-down position, the changes in pressure were considerably greater than in the intact animal. These experiments are taken to indicate that the intact character of the vertebral and cranial bony system is essential for the adequate protection of the central nervous system against alterations in pressure in the cerebrospinal fluid, on tilting from the horizontal to the vertical position.

AUTHORS' SUMMARY.

SPONTANEOUS SUBARACHNOID HEMORRHAGE. W. R. OHLER and D. HURWITZ, J. A. M. A. **98**:1856, 1932.

Spontaneous subarachnoid hemorrhage, which occurs more frequently than is generally understood, comes on suddenly, usually with headache, occasionally with vomiting, dizziness, stupor or coma, and rarely with convulsions. Stiff neck, Kernig's sign, fever and slight leukocytosis may lead to confusion of this condition with meningitis. Hypertension is present frequently.

PERMEABILITY OF THE SKIN VESSELS. P. D. McMASTER and S. HUDACK, J. Exper. Med. **55**:417 and 431, 1932.

The gradient of permeability that exists along the cutaneous capillaries and venules is accentuated and broadened in scope by increasing the venous pressure moderately. Under such circumstances, transudation leading to edema takes place most abundantly from the venules. The permeability of the portion of the capillary web that is near the arterioles increases only when the venous pressure rises so high as to approximate that in the arteries. Under such circumstances, the gradient of permeability along the small vessels disappears, the capillaries and venules everywhere leaking fluid. The character of the vital staining under such circumstances indicates, like the evidence of previous work, that the gradient results from structural differentiation.

The mounting gradient of permeability along the small vessels of the corium is essentially unaltered by active hyperemia produced by heat, cold or light. Only when the vascular walls are so damaged that rapid leakage ensues, as shown by edema, does the permeability of the capillary web as a whole approximate that of the venules. It is plain that the normal gradient of vascular permeability depends on the integrity of the wall of the blood vessel. The method of experiment described can be utilized for a study of the functional changes that result in lesions due to burning and freezing.

AUTHORS' SUMMARY.

THE AGE FACTOR IN THE VELOCITY OF THE GROWTH OF FIBROBLASTS IN THE HEALING WOUND. E. L. HOWES and S. C. HARVEY, J. Exper. Med. **55**:577, 1932.

The velocity curve of fibroplasia in the healing of wounds in young rats reached its end-point three days ahead of a similar curve for adults. Strength and fibroplasia were manifest one day sooner than in the adults. A study of the increments of the curve showed that the rate of fibroplasia during the accelerated phase was less in the young, and that it lasted longer. Correspondingly, retardation appeared later and was less than in the curve for the adult rats. The retardation was even less than in the curve for adult rats on a high protein diet, in spite of the fact that in the latter curve there was a definite increase in the rate of fibroplasia. Healing in the young, therefore, is more rapid than in adults because fibroplasia begins earlier and is less retarded, not because the rate of fibroplasia is greater. Growth of the young is not hindered by the process of wound healing.

AUTHORS' SUMMARY.

RENAL THRESHOLDS FOR HEMOGLOBIN IN DOGS. J. A. LICHTY, JR., W. H. HAVILL and G. H. WHIPPLE, J. Exper. Med. **55**:603, 1932.

We use the term "renal threshold for hemoglobin" to indicate the smallest amount of hemoglobin that given intravenously will effect the appearance of recognizable hemoglobin in the urine. . . . Rest periods without injections cause a return of the renal threshold for hemoglobin toward the initial level—a recovery level. Injections of hemoglobin below the initial, but above the minimal, or depression, threshold level will eventually reduce the renal threshold for hemoglobin to its depression level. We believe that the depression, or minimal, renal threshold level due to repeated injections of hemoglobin is a little above

the glomerular threshold, which we assume is the base line threshold for hemoglobin. Our reasons for this belief in the glomerular threshold are given in this paper and in the other papers of this series.

AUTHORS' SUMMARY.

FAT MOBILIZATION IN STARVATION. J. H. DIBLE, *J. Path. & Bact.* **35**:451, 1932.

The degree of fat infiltration in the liver in starved rats is dependent on the quantity of fat available for mobilization in the animals' storage depots. Once the metabolism of starvation is established, this factor is the determining one and is independent of time. The histologic changes, acidosis and changes in the non-saponifiable extractable material are discussed in their relationship to these findings.

AUTHOR'S SUMMARY.

EXPERIMENTAL ATHEROSCLEROSIS OF THE CORONARY ARTERIES OF RABBITS. K. WOLKOFF, *Beitr. z. path. Anat. u. z. allg. Path.* **85**:386, 1930.

On feeding of cholesterol to rabbits, Wolkoff demonstrated, after from 139 to 176 days, progressive focal subendothelial deposition of lipoids, which predominated about the origins of the coronary vessels and at their points of division. After the longer periods there were associated proliferation of the fixed cells, increase of elastic and collagenic tissue and presence of "xanthoma" cells. The media contained a little interstitial lipoid.

In rabbits studied from 100 to 1,003 days after cessation of the cholesterol feeding, there was noted gradual disappearance of the lipoid deposits with formation of intimal plaques composed of hyalinized collagenic fibers in which were embedded lipoid droplets, cholesterol crystals and calcium granules. The media underneath the plaques was frequently secondarily atrophied to almost complete disappearance.

Wolkoff considers this experimental coronary atherosclerosis, as regards both histologic aspects and distribution, very similar to the human disease, and therefore assumes the pathogenesis of both to be identical.

W. S. BOIKAN.

RELATIONSHIP BETWEEN CARBOHYDRATE METABOLISM AND MITOCHONDRIA IN THE LIVER. K. TANIGUCHI and M. HIKIMA, *Beitr. z. path. Anat. u. z. allg. Path.* **85**:565, 1930.

The normal lactic acid content of the blood was determined as from 20 to 28 mg. per cent. A rise to 30 mg. per cent in human subjects was correlated by biopsy with definite morphologic alterations in the mitochondria and in their distribution in the liver. The determination of lactic acid in the blood is therefore considered a sensitive and exact test for hepatic function.

W. S. BOIKAN.

RETINAL RESPIRATION AND AMINO-ACIDS. BRUNO KISCH, *Biochem. Ztschr.* **244**:459, 1932.

In contrast with tumor tissue, retina shows a definite respiratory increase on addition of glycocoll, alanine and phenylalanine. This effect is more marked in old than in fresh retinal tissue. In distinction from renal tissue, retinal responds less markedly to alanine and phenylalanine with respiratory increase than to serine, valine and sarcosine.

WILHELM C. HUEPER.

EXPERIMENTAL PRODUCTION OF FIBROUS OSTEITIS. E. RUTISHAUSER, *Centralbl. f. allg. Path. u. path. Anat.* **53**:305, 1932.

Human parathyroid glands were roughly sterilized in chloroform, macerated in salt solution and injected into guinea-pigs subcutaneously. In the majority of the animals abscesses developed, but Rutishauser feels that he has enough data

to summarize as follows: Fibrous osteitis can be produced in guinea-pigs by the injection of macerated human parathyroid tissue. The animals have an elevated calcium content of the blood. It is possible to see the first stages of the disease after thirty days; these are characterized by resorption processes and the formation of fibrous marrow. Suppuration does not cause the disease, because the picture can be duplicated by the subcutaneous injection of 3.5 Gm. of dextrose twice daily for about one hundred days. Schmidtman has produced it by the subcutaneous injection of ammonium chlorate and by the administration of viosterol.

GEORGE RUKSTINAT.

THE METABOLISM OF THE SUPRAVITAL NORMAL AND GOITROUS THYROID GLAND. B. WALTHARD, *Ztschr. f. d. ges. exper. Med.* **79**:451, 1931.

The metabolism of the normal thyroid gland of the wild rat and of different types of human goiter was studied by the method of Warburg. A simultaneous histologic examination of every specimen appeared to be valuable, because degenerative changes may influence the metabolism in the same way as does cell proliferation. The metabolism of the normal and the goitrous thyroid gland was found to be governed by the laws of Warburg. Normal thyroid tissue showed a high grade of oxidation and no aerobic fermentation of sugar. Diffuse goiters behaved like normal tissue as long as degenerative changes were absent. In parenchymatous adenomas (so-called fetal adenomas) a metabolic effect was observed typical for benign tumors, namely, high rate of oxidation and aerobic fermentation of sugar. Degenerative changes, as recognized histologically, were accompanied by decrease in respiration and increased aerobic glycolysis. The malignant goiters reacted like other cancerous growths, showing marked fermentation and diminished oxidation.

C. A. HELLWIG.

### Pathologic Anatomy

CONTRACTED KIDNEYS IN CHILDHOOD. M. KLEIN, Frankfurt. *Ztschr. f. Path.* **41**:317, 1931.

The author describes one case of malignant nephrosclerosis and two cases of chronic glomerulonephritis (secondary contracted kidneys) occurring in children. A short review of reported cases of malignant nephrosclerosis is also given. The differences between the two types of kidneys are discussed. In cases of malignant nephrosclerosis, endarteritis and periarteritic changes combined with necrosis of the walls of the arterioles are found. These changes extend into the vasa afferentia and capillaries of the glomeruli. In cases of chronic glomerulonephritis, blood vessel changes may be present, but endarteritis and arterionecrosis are not characteristic. In cases of malignant nephrosclerosis, inflammatory changes within the glomeruli may be the result of primary inflammatory changes of the blood vessels. The inflammatory changes of the glomeruli are not so severe and diffuse as in cases of chronic glomerulonephritis. The arteriolosclerosis leads to disappearance of glomeruli, without accompanying inflammatory changes of the glomerulus. Malignant nephrosclerosis does not occur secondary to "nephrocirrhosis arteriolosclerotica." The author believes that there are only nine proved cases of malignant nephrosclerosis in children on record, while twenty-four cases that have been reported to be such are doubtful. Chronic inflammatory contracted kidneys (chronic glomerulonephritis) in children was found described in the literature twenty-two times. The blood vessel changes that are found in the spleen, liver, pancreas, brain, thymus and suprarenal glands are not inflammatory. Morphologically, they resemble the changes seen in arteriosclerosis. They probably are the result of the increased pressure occurring in renal insufficiency.

O. SAPHIR.



THE PATHOGENESIS OF A RARE MALFORMATION OF THE HEART (COR TRIA-TRIATUM). J. HAGENAUER, Frankfurt. Ztschr. f. Path. **41**:332, 1931.

Cor triatriatum in a 4 months old child is reported. The heart was enlarged owing to marked hypertrophy of the right ventricle; the left ventricle was small and gave the impression of being an appendage to the right ventricle. The right auricle was dilated and revealed the normal openings of the superior and inferior vena cavae. The interauricular septum showed an opening that led from the right into a small left auricle. This left auricle had no communication with the left ventricle, but formed a small blind-ending sac. The four pulmonary veins, however, opened into this auricle. When the left ventricle was opened, it was noted that it led through a normal mitral valve into a second left auricle. This second auricle revealed no communications with the first left auricle or with the pulmonary veins. A small auricular appendage, however, was attached to it. An open foramen ovale led from the second left auricle into the right auricle. The blood stream apparently flowed from the pulmonary veins into the first left auricle and from there through the open intra-auricular septum into the right auricle. Some of the blood passed into the right ventricle and some through the open foramen ovale into the second left auricle and from there into the left ventricle and the aorta. The possible conditions leading to this rare malformation are discussed. The underlying cause is apparently a secondary occlusion of the pulmonary vein, which primarily was open.

O. SAPHIR.

THE MECHANISM OF THE FORMATION OF CARDIAC THROMBI. M. PLONSKIER, Frankfurt. Ztschr. f. Path. **41**:357, 1931.

In a 6 year old girl, autopsy revealed endocarditis of the tricuspid and pulmonary valves. In addition, an open interventricular septum was found. The endocardium of the right ventricle in an area just opposite the septal defect revealed a thrombus. The author believes that the force of the blood stream coming from the open interventricular septum and directed on the right ventricular endocardium, combined with the force of the normal systolic blood stream, led to the production of endocardial thickenings in a circumscribed area. The thickened endocardium became a fitting location for the thrombus. Because of the fact that bacteria were found only on the surface of the thrombus, and not in the midportion or base, the author does not believe that a localization of bacteria was responsible for the formation of the thrombus. He is of the opinion that cardiac weakness in the course of a severe infectious disease causing slow circulation led to the formation of the thrombus in an area that was predisposed to such formation by endocardial changes.

O. SAPHIR.

CIRCUMSCRIBED OSTEOPOROSIS OF THE CRANIUM. W. SCHELLENBERG, Frankfurt. Ztschr. f. Path. **41**:423, 1931.

In a 40 year old man, the clinical diagnosis was: frontal sinusitis, with erosion and perforation of the frontal bone, and collection of air extradurally. An operation was performed to remove the air. The patient died shortly afterward. At autopsy, a cyst the size of a goose egg was present in the brain substance between the right parietal and occipital lobes. The cyst, which was filled with a clear liquid, did not communicate with the ventricular system. The left portion of the skull was much softer than the right. In the former, differentiation between the external and internal tables and the diploe was very indistinct. Histologic examination of these portions revealed circumscribed osteitis fibrosa. The relation between this disease and Paget's disease is discussed. The cyst in the brain is regarded as a "tied-off (*abgeschnürte*) ventricular cyst."

O. SAPHIR.

THREE CASES OF SPECKLED SPLEEN (MULTIPLE NECROSIS OF THE SPLEEN),  
WITH SPECIAL REFERENCE TO CHANGES IN THE KIDNEYS. E. ADOLPHS,  
Frankfurt. Ztschr. f. Path. **41**:435, 1931.

In three cases of speckled spleen, many areas of necrosis were found in the splenic tissue, caused by diffuse arteriolonecrosis. While in two cases all of the larger arteries of the spleen also showed simple arteriosclerosis, in the last case only arterioneclerosis was revealed. In all three cases, severe lesions of the kidneys were found. In the first two instances, there was also chronic glomerulonephritis, with arteriolonecrotic changes corresponding to those seen in malignant sclerosis or malignant arteriolonecrosis of the kidney. These findings are compared with those in simple arteriolosclerosis of the kidney. Even though in both conditions (arteriolonecrosis and arteriolosclerosis) hyalinization and lipid degeneration are present in the arterioles, the appearance of a fibrinous exudate extending to the adventitia and to the surrounding tissues, necrosis and the presence of polymorphonuclear leukocytes are characteristic of arteriolonecrosis. The lumina of the arterioles in arteriolosclerosis are small or obliterated. In arteriolonecrosis, however, they may be wider than normal, probably because of the pressure exerted by the blood on the necrotic walls of the vessels. The question of whether arteriolonecrosis is the sequel of arteriolosclerosis is discussed. Because of the finding of arteriolonecrosis in the kidney in the third case without arteriolosclerosis, a relationship between these conditions seems questionable. The second case also showed a very recent arteriolonecrosis of all organs, especially of the skin, tongue, myocardium intestines and spleen. Some similarity of these diffuse lesions and those found in periarteritis nodosa is also discussed. The author believes that arteriolonecrosis occurs especially in kidneys showing chronic inflammatory lesions. The changes in the spleen are similar to those found in the kidneys. They are the result of arteriolonecrosis. In regard to the etiology of the lesions of the blood vessel, the author is inclined to believe in the hypothesis of Ricker, who stated that the necrosis of the wall of the blood vessel is the result of a marked irritation of the vasomotor nervous mechanism, with resulting paralysis of the walls. The necrosis is caused by the stoppage of the circulation of the tissue fluids in the walls.

O. SAPHIR.

ADIPOSOGENITAL DYSTROPHY. H. HEINRICHS, Frankfurt. Ztschr. f. Path.  
**41**:512, 1931.

In a 70 year old man, there was marked fibrosis of the capsule of the anterior lobe of the hypophysis. The fibrosis extended into this lobe and had led to a partial destruction of the tissue. The patient showed not only pituitary nanosoma but also the characteristic picture of adiposogenital dystrophy, with retardation of growth and hypoplasia of the testes.

O. SAPHIR.

ANATOMIC CHANGES OF HEART AND BLOOD VESSELS IN SEPTIC SCARLATINA.  
H. SIEGMUND, Verhandl. d. deutsch. path. Gesellsch. **26**:231, 1931.

In fifty-six autopsies on children who had died during an epidemic of scarlet fever, typical nodular infiltrations were found in the endocardium and in the walls of veins of internal organs, namely, spleen, liver, kidneys and suprarenal glands, in 90 per cent. In the aorta, perivascular infiltration of the vasa vasorum was often present. In those who died about eight weeks after the onset of the disease, nodules were found in the heart muscle and in the endocardium which had the same structure as the rheumatic bodies of Aschoff. The same changes were noticed in the peritonsillar tissue. The author regards these lesions as allergic reactions of a sensitized organism against streptococcal infection. In all his cases, hemolytic streptococci were cultivated from the blood at autopsy.

C. ALEXANDER HELLWIG.

RETICULO-ENDOTHELIAL SYSTEM AND AMYLOID. H. J. ARNDT, *Verhandl. d. deutsch. path. Gesellsch.* **26**:243, 1931.

In 100 horses used in the production of antitoxin at the Behring works at Marburg, Arndt and Doerken studied the morphologic aspect of immunobiologic processes, especially the behavior of the reticulo-endothelial system and the formation of amyloid. In the livers of the horses characteristic changes were observed: diffuse swelling of the endothelial cells, nodular granulomas and endophlebitic proliferation. These changes suggest that the antibodies are formed in the reticulo-endothelial system. In 60 of the 100 horses amyloid was demonstrated, most frequently in the spleen, less often in the liver and only rarely in other organs. Chemical analysis of the amyloid gave results similar to those obtained by analysis of human amyloid. There seems to be a relationship between the formation of the amyloid and the reaction of the reticulo-endothelial cells. The latter is regarded as the initial stage of amyloidosis. On the other hand, resorption of amyloid by reticulo-endothelial cells is suggested by the histologic observations.

C. ALEXANDER HELLWIG.

### Pathologic Chemistry and Physics

PULMONARY GAS ABSORPTION IN BRONCHIAL OBSTRUCTION. P. N. CORYLLOS and GEORGE L. BIRNBAUM, *Am. J. M. Sc.* **183**:317, 1932.

Experimental methods have been devised which give evidence that when a bronchus is completely obstructed the entrapped alveolar air rapidly undergoes qualitative and quantitative changes as determined by successive gas analyses. Qualitatively, the percentages and partial pressures of the gases comprising the alveolar air tend to, but never reach, an equilibrium with the gases of the venous blood. Quantitatively, the entrapped alveolar gases pass through the respiratory membrane into the blood circulating in the peri-alveolar capillaries until complete airlessness of the involved area is produced. The mechanism of production of atelectasis in the compressed lung (pneumothorax, pleural exudate, intrathoracic tumors, etc.) is exactly the same as in bronchial obstruction. Besides the gases of the air, diffusion of other gases was studied by introducing them into a lung previously rendered atelectatic. The different gases used in these experiments were: (a) active gases, oxygen and carbon dioxide; (b) neutral gases, hydrogen, nitrogen and helium; (c) anesthetic gases or vapors, ether, ethyl chloride, nitrous oxide and ethylene. The new experimental methods allow direct vision of the pulmonary changes occurring during the experiment. Nitrogen in the respiratory air plays the part of a "mechanical buffer," retarding the absorption of more diffusible and more soluble gases. This experimental work has allowed the formation of a theory on the mechanism of atelectasis based on the physiology of exchange of gases in the lung.

AUTHORS' SUMMARY.

WATER AND OTHER INORGANIC CONSTITUENTS IN THE HEART MUSCLE OF TUBERCULOUS PATIENTS. L. C. SCOTT, *Am. Rev. Tuberc.* **23**:429, 1931.

The principal inorganic constituents of fourteen hearts from tuberculous patients agree fairly well with those of an equal number of hearts from persons dying from various other diseases with the sole exception of the water content. The latter is sufficiently in excess of the average found in other diseases to warrant the assumption that the excess is characteristic of this disease, especially when the disease has been of comparatively long standing.

H. J. CORPER.

A METHOD FOR THE DETERMINATION OF THYRONINE IN THE THYROID. J. P. LELAND and G. L. FOSTER, *J. Biol. Chem.* **95**:165, 1932.

An improved method is described for the isolation and estimation of the thyroxine content of human thyroid tissue. Approximately 25 per cent of the total iodine of fifty-two glands was present as thyroxine.

E. R. MAIN.

NORMAL PLASMA PROTEIN CONCENTRATION IN SPITE OF LOSS BY BLEEDING. C. W. BARNETT, R. B. JONES and R. B. COHN, *J. Exper. Med.* **55**:683, 1932.

From 25 to 100 cc. of blood plasma was removed daily from five dogs, the red cells being returned to the circulation in Locke's solution. In no case was there a significant drop in the protein concentration of the plasma. A gravimetric method for the determination of the total amount of protein in the plasma is described. A case of cirrhosis of the liver is reported in which over 10 Gm. of protein was lost daily in the ascitic fluid during a period of seven months without any lowering of the protein concentration of the plasma. The constancy of the protein level and the adequacy of the mechanism of regeneration are pointed out.

AUTHORS' SUMMARY.

THE BIOLOGIC ACTION OF ULTRAHIGH FREQUENCY CURRENTS. R. A. HICKS and W. T. SZYMANOWSKI, *J. Infect. Dis.* **50**:466, 1932.

For the moment, no studies in this field can be regarded as final, especially with respect to the interpretation of the results. If one considers the gamut of wavelengths, each one of which may offer a distinctive action, if one considers the enormously variable factors in thermal conditions, character of medium, period of exposure, potential across the plates and different conditions of tuning, the problem, from the point of view of its physical set up, becomes very extensive. If, in addition, one considers the number of bacterial variants, as well as other biologic substances that might be studied under the whole scale of physical conditions possible, it becomes obvious that no final statement may be made with regard to the biologic action of ultrahigh frequency currents. It is possible, however, to say from the work now reported that at least there is no biologic action on the substances mentioned under the described conditions of exposure to ultrahigh frequency fields. It is also probable that indefinite extension of this study may not lead to a vigorous and readily detectable direct biologic action without some marked technical advance beyond that of the present day. It must be borne in mind, however, that almost infinite possibilities exist for the development of this field, and accordingly negative conclusions at this time are restricted to the experimental conditions employed.

AUTHORS' SUMMARY.

ERGOTHIONEINE CONTENT OF THE BLOOD IN HEALTH AND DISEASE. H. B. SALT, *Biochem. J.* **25**:1712, 1931.

An improved method is described for the estimation of ergothioneine in blood. Ergothioneine is not present in the blood plasma of normal persons. From 3 to 12 mg. per hundred cubic centimeters is found in the erythrocytic fraction of blood obtained from normal persons and from persons with diseases other than diabetes. Slightly higher values are observed in diabetes.

E. R. MAIN.

BLOOD PHOSPHATASES. JEAN ROCHE, *Biochem. J.* **25**:1724, 1931.

The phosphatase in the erythrocytes appears to differ from that in the serum and leukocytes. The latter may be identical with the phosphatase of the kidneys and intestines. Phosphatases hydrolyze mono-substituted glycerophosphates, but have no appreciable action on di-substituted esters. They synthesize phosphoric esters from inorganic phosphate and the various alcohols.

E. R. MAIN.

FERMENTABLE SUGAR IN NORMAL URINE. V. J. HARDING and D. L. SELBY, *Biochem. J.* **25**:1815, 1931.

Sugar capable of being fermented by yeast is not present in the urine of normal persons following a twelve hour fast. Small amounts may be present immediately

following the ingestion of fructose or large quantities of fruit. Although glycosuria does not occur following the ingestion of 50 Gm. of dextrose in the early morning after a twelve hour fast (the dextrose tolerance test), it may occur following the ingestion of sugar at 4 p. m.

E. R. MAIN.

SPERMINE IN HUMAN TISSUES. G. A. HARRISON, *Biochem. J.* **25**:1885, 1931.

The human prostate gland contains more than 300 mg. of spermine phosphate per hundred grams of tissue. The concentration of the base per hundred grams of seminal plasma is approximately 100 mg. From 1 to 9 mg. per cent is present in the testis, approximately 16 is in the pancreas, from 3 to 8 in the spleen, from 5 to 6 in the kidney, from 2 to 11 in the liver, from 3 to 5 in the lung, from 2 to 3 in the brain and from 1.5 to 2 in the heart. A trace of spermine was observed in the dried feces; none was found in the bone-marrow, blood or sputum. The derivation of spermine from the prostate gland, rather than from the testicle, is confirmed by the absence of the substance in the semen of the skull. The bull does not have a truly functional prostate gland.

ARTHUR LOCKE.

AN IN VITRO EFFECT OF ANTI-NEURITIC VITAMIN CONCENTRATES. N. GAVRILESCU and R. A. PETERS, *Biochem. J.* **25**:2150, 1931.

The amount of oxygen absorbed by finely minced preparations of the lower parts of the brains of normal pigeons is increased approximately 40 per cent when the measurements are made in an atmosphere of oxygen in place of air. The amount of oxygen absorbed by similar preparations of brain from avitaminous ( $B_1$ ) pigeons is not only less than normal, but is markedly less influenced by the oxygen content of the atmosphere in which the measurements are made. This curious difference is less manifest when the preparations are admixed, in vitro, with a concentrate containing vitamin  $B_1$ . The restorative action of the vitamin appears to be catalytic and not due to substances other than the vitamin adventitiously present in the concentrate. Vitamin  $B_1$  appears to be directly concerned in the regulation of the respiration and function of the lower parts of the pigeon brain.

ARTHUR LOCKE.

A PIGMENT IN THE SWEAT AND URINE OF CERTAIN SHEEP. C. RIMINGTON and A. M. STEWART, *Proc. Roy. Soc., London*, s. B. **110**:75, 1932.

The golden-brown coloration of the wool fiber of many sheep appears to be due to a pigment, lanaurin, which is secreted in the sweat. The pigment may be isolated from both the wool and the urine. It is a pyrrolic complex and probably occupies an intermediate position between the bile pigments and fully condensed melanin. The condition in sheep resembles familial acholuric jaundice in man. Both phenomena may be due to a hereditary tendency to hyperactivity on the part of the cells of the reticulo-endothelial system, with increased destruction of hemoglobin. Lanaurin may be considered to be derived from hemoglobin and to be excreted by the renal and sudorific systems.

E. R. MAIN.

COLORIMETRIC  $p_H$  OF MALIGNANT CELLS IN TISSUE CULTURE. R. CHAMBERS and R. J. LUDFORD, *Proc. Roy. Soc., London*, s. B. **110**:120, 1932.

The intracellular hydrogen ion concentration of mouse tumors in culture is  $p_H 6.8 \pm 0.1$ , a value comparable to that observed in normal cells of a similar nature. After injury or cytolysis, the  $p_H$  value of both tumor and normal cells decreases to 5.6 or less. The intranuclear hydrogen ion concentration is  $p_H 7.2$  and does not appear to be lowered following injury.

E. R. MAIN.

RETENTION OF MERCURY IN THE BODY. HOLTZMANN, Arch. f. Hyg. **106**:377, 1931.

Mercury was found at autopsy in the lungs of guinea-pigs poisoned by the continued respiration of air containing droplets of this metal. Appreciable amounts were found in the kidneys, through which it is slowly eliminated. Much smaller amounts were found in the liver.

ARTHUR LOCKE.

SIGNIFICANCE OF COPPER, ZINC AND MANGANESE IN PATHOLOGY. W. HERKEL, Beitr. z. path. Anat. u. z. allg. Path. **85**:513, 1930.

By prolonged feeding of various copper salts to rabbits and rats, Herkel could produce increased storage of copper in the liver, but not cirrhosis. He found increased copper in the liver not only in patients with hematochromatosis but also in those with nonpigmentary cirrhosis, in pregnant women and in nursing infants. The need of rapidly growing tissues for copper is suggested as the possible explanation. Zinc values showed no significant variations.

W. S. BOIKAN.

OCCURRENCE OF GLYCOGEN IN ADIPOSE TISSUE. F. RICHTER, Beitr. z. path. Anat. u. z. allg. Path. **86**:65, 1931.

Glycogen can be demonstrated in the fat tissue of the white rat if, after preliminary starvation, carbohydrates are fed. It represents an intermediate stage in the synthesis of fat. But the reverse—the appearance of glycogen during the utilization of fat—does not occur. Epinephrine and insulin do not lead to storage of glycogen in the fat tissue. The glycogen content of fetal tissue is independent of the maternal. The fat tissue of fetal rats as well as that of the human cadaver contains no glycogen.

W. S. BOIKAN.

POTASSIUM CONTENT OF BONE MARROW IN CANCER. T. HOFFMANN, Biochem. Ztschr. **243**:145, 1931.

The quantitative analysis of bone marrow during the age of growth showed a high content of potassium, dropping to from one fourth to one fifth of this value after the end of this period. In the presence of carcinoma, a new accumulation of potassium in the bone marrow is seen. The values obtained are at least twice as large as those for the normal, noncarcinomatous adult. The sodium content of the bone marrow shows similar, but less constant, variations. The variations in the calcium content have no relations to the growth.

WILHELM C. HUEPER.

PHENOL DERIVATIVES IN URINE. OTTO FUERTH and RUDOLF SCHOLL, Biochem. Ztschr. **243**:276, 1931.

Three types of phenol derivatives may occur in the urine: (1) substances that can be removed by steam distillation, such as phenol and cresol; (2) substances that can be extracted with ether, such as *p*-oxyphenyl propionic acid, *p*-oxyphenyl acetic acid, *p*-benzoic acid, *p*-oxyphenyl lactic acid and *p*-oxyphenyl pyruvic acid (which are derivatives of tyrosine through deamidization); (3) substances which are neither ether-soluble nor removable by steam distillation, and which are designated as oxyproteinic acids, contributing to the diazo reaction. Also chromogen belongs in this group.

Causes for their appearance in the urine are: (1) increased putrefaction of protein in the intestine, (2) disturbed function of the liver (acute yellow atrophy, phosphorus poisoning, etc.) and (3) increased decomposition of protoplasm in the living organism.

The phenol fraction that can be distilled off is clinically unimportant. The ether-soluble fraction can be determined colorimetrically, if a larger amount of urine (1,000 cc.) is acidified with sulphuric acid till congo red paper turns blue,

and then concentrated in a vacuum (35 mm. of pressure) and nitrogen current at low temperature to 100 cc., and finally shaken twice with 25 cc. of ether. The ether is washed twice with water and then shaken with a small amount of 10 per cent sodium hydroxide, which extracts the phenol substances. The aqueous layer is acidified with 50 per cent sulphuric acid, and after brief boiling, decolorized with charcoal and examined with Millon's reagent colorimetrically. A 1 per cent tyrosine solution in 5 per cent sulphuric acid is used as a standard. The normal human urine contains between 0.0043 to 0.0050 mg. of phenol per hundred cubic centimeters, calculated as tyrosine. The ether-insoluble fraction can be determined in the urea-free fraction of the urine, which is obtained after the Mörner-Sjöquist method by precipitation with a solution of barium hydrate (15 Gm. in an alcohol-ether mixture—ratio 2:1). The barium hydrate is removed by sulphuric acid; interfering substances, by tungstic acid; these again are removed by the addition of quinine in excess, which in its turn is removed by the addition of sodium hydroxide. The phenol can then be determined with Millon's reagent. Normal urine contains only traces of these substances, which cannot be determined colorimetrically. The determination of indican in the urine by the method of Wang was found to be unreliable. The normal indican content of human urine estimated with the colorimetric method, with sodium indigo-sulphonic acid used as a standard, varies between 0.8 and 1.3 mg. per hundred cubic centimeters. The amount of ether-soluble phenol remains within normal limits in pathologic urine, while the amount of the ether-insoluble fraction increases considerably in certain conditions, reaching values up to from 36 to 104 mg. per hundred cubic centimeters (acute yellow atrophy). Similar values were obtained in cases with functional disturbances of the liver (in catarrhal jaundice, but not in cirrhosis), in severe intestinal disturbances, and in advanced tuberculosis with positive urochromogen reaction. In schizophrenic patients, only exceptionally increases of the ether-insoluble fraction were seen. There is no relation between the urinary indican due to putrefaction of protein and the ether-insoluble fraction of phenols.

WILHELM C. HUEPER.

TITRIMETRIC DETERMINATION OF AMINO-ACIDS IN BLOOD SERUM. KONRAD L. ZIRM and JOHANN BENEDICT, *Biochem. Ztschr.* **243**:312, 1931.

Three cubic centimeters of serum is deproteinized with colloidal iron hydroxide under heating. The hot fluid is centrifugated, and the clear supernatant fluid is evaporated over a water bath. The dried material is dissolved in water, and after addition of L-naphthyl red it is titrated against a water control with a fortieth-normal alcoholic hydrochloric acid solution. By this method, the strongly basically reacting nitrogen-containing groups are determined. If instead of naphthyl red, 2, 4, 2', 4', 2'' pentamethoxytriphenylcarbinol is used, a preliminary titration of the control can be omitted. Normal serums have an amino-nitrogen level of from 5.4 to 8 mg. per hundred cubic centimeters. The Van Slyke method gives slightly higher values, owing to partial inclusion of urea nitrogen in the values obtained. The results agree well with those obtained by the Folin method.

WILHELM C. HUEPER.

## Microbiology and Parasitology

THE EFFECT OF TESTICULAR PASSAGE ON THE VIRUS OF HERPES. W. SMITH, *J. Path. & Bact.* **34**:747, 1931.

The power of causing specific adrenal lesions and cutaneous reactions in rabbits is probably common to all strains of herpes virus, the production of such lesions being dependent on the quantity of virus inoculated. The testis of the rabbit provides a better culture medium for the growth of herpes virus than the brain; this holds true even for intensely neurotropic strains. The nonspecific factor

described by Duran-Reynals as enhancing the virulence of vaccinia virus does not account for the greater infectivity of herpes testis as compared with herpetic brain.

AUTHOR'S SUMMARY.

EXPERIMENTAL POLIOMYELITIS FROM INTRATHECAL INOCULATION OF THE VIRUS.  
E. W. WESTON, *J. Path. & Bact.* **35**:41, 1932.

After intrathecal inoculation of poliomyelitic virus, the distribution of both the virus and the lesions in the cerebral hemispheres is strikingly different from that of the maximum meningitis or of the deepest staining with dyestuffs injected into the fluid. Frequently the earliest lesions are situated in the floor of the fourth ventricle, into which under the conditions of the experiment the virus regurgitates at operation, and the influence of axonic transmission in their subsequent spread is again evident. It is suggested that penetration of the nerve tissues may occur through the ependyma of the fourth ventricle, but an attempt to demonstrate the permeability of a purely meningeal surface was unsuccessful. Considering the pathogenesis of human poliomyelitis, it is concluded that no evidence available speaks against an axonic entry of the virus or necessitates the participation of the cerebrospinal fluid in its spread through the nervous system.

AUTHOR'S SUMMARY.

THE BLOOD SUGAR AND PHOSPHORUS IN RABBITS AFTER THE INJECTION OF  
SUSPENSIONS OF DEAD BACTERIA. M. E. DELAFIELD, *J. Path. & Bact.*  
**35**:53, 1932.

Intravenous injections of suspensions of dead gram-positive organisms into rabbits produced no obvious illness. Ten different organisms were used. With the exception of *Streptococcus pneumoniae*, which caused a fall in the organic phosphorus of the blood, none of the organisms evoked any significant change in the sugar or the phosphorus level. Eight of the twelve gram-negative organisms tested produced obvious illness of the animals, with hyperglycemia and low inorganic phosphorus at two hours after injection, followed at twenty-four hours by a lower level of sugar and a higher level of phosphorus. Two of the twelve gram-negative organisms produced the chemical response without illness. Two of the twelve gram-negative organisms produced no illness and no typical chemical response. *Staphylococcus aureus* and *Strep. viridans*, rendered in part gram-negative by long growth in broth, were inert as were also the bacterial filtrates of these organisms. There appears to be a significant correlation between the changes in the sugar and the phosphorus content of the blood in the first twenty-four hours after injection. High inorganic phosphorus is always found when the animal is gravely ill at any time subsequent to the first twenty-four hours after injection.

AUTHOR'S SUMMARY.

RELATIVE INCIDENCE OF HUMAN AND BOVINE TUBERCLE BACILLI IN TUBERCULOUS MENINGITIS IN ENGLAND. A. S. GRIFFITH, *J. Path. & Bact.*  
**35**:97, 1932.

Cultures of tubercle bacilli have been obtained from the cerebrospinal fluid in thirty new cases of tuberculous meningitis (years 1930 and 1931), twenty-nine of which occurred in children under 10 years and one in an adult. Of the twenty-nine children, twenty were infected with bacilli of bovine type. The adult was also infected with bovine bacilli. Twenty-seven of the cases occurred in Leeds, and in eight (29.6 per cent) of these cultures yielded bovine tubercle bacilli. Of the remaining cases due to infection with bovine bacilli one was derived from Lancashire and one from Lincolnshire. The total number of cases of tuberculous meningitis in which cultures have been obtained from cerebrospinal fluid drawn during life is sixty-three. Of these nineteen (30.2 per cent) were due to bovine bacilli. With this percentage as a basis it is estimated that in England and



Wales upward of seven hundred persons died of tuberculosis of the central nervous system caused by bovine tubercle bacilli in each of the three years 1928, 1929 and 1930.

AUTHOR'S SUMMARY.

SCARLATINAL STREPTOCOCCI IN NONSCARLATINAL INFECTIONS. V. D. ALLISON, *Lancet* 2:844, 1931.

Of 396 strains of hemolytic streptococci isolated from nonscarlatinal infections, only 8 (2 per cent) were found to belong to one or other of the four main serologic types of scarlatinal streptococci. The results indicate the high degree of specificity of the scarlatinal streptococci, as 63 per cent of hemolytic streptococci from scarlatinal patients can be typed serologically. Among the 8 typed strains, a type I strain and a type III strain were isolated from uterine cervical swabs in cases of puerperal infection, while a type I strain and a type III strain were isolated from throat swabs of midwives in attendance on the respective patients. These results corroborate those recently shown by Smith and support the view of many clinicians that puerperal infection may arise by infection from the air passages of persons in attendance on the patients. In 100 consecutive cases of diphtheria and 100 consecutive cases of measles, 62 strains and 32 strains, respectively, of hemolytic streptococci were isolated, but none were placed in any of the main serologic types of scarlatinal streptococci.

AUTHOR'S SUMMARY.

THE ULTRAVIRUS OF TUBERCULOSIS. F. VAN DEINSE, *Ann. Inst. Pasteur* 47:135, 1931.

The ultravirus, when inoculated into the living guinea-pig, develops rapidly in pus formed within the peritoneal cavity. Calcium phosphate seems to aid in the development if injected several days after the introduction of tuberculous filtrate. Acid-fast organisms may be demonstrated in animals killed from two to four days after inoculation. The observations are offered, incidentally, as a means of differentiating the ultravirus from the visible bacilli.

M. S. MARSHALL.

THE ETIOLOGY OF ULCERATIVE COLITIS. R. BUTTIAUX and A. SÉVIN, *Ann. Inst. Pasteur* 47:173, 1931.

An extensive study of ulcerative colitis is concluded with the following résumé: ". . . We think that the infectious etiology of ulcerative colitis should be interpreted in the following manner. There exist forms of chronic colitis that arise from specific organisms of this disease. These organisms are rare; only two of them have been recognized, Barger's organism (a diplococcus) and ours (also a diplococcus).

"Other forms of ulcerative colitis which we designate as 'recalled' (de rappel) are due to the awakening of organisms that have previously provoked a general infection, cured at the moment when intestinal damage occurs.

"A third category of colitis which we designate as 'de sortie' by analogy to that which occurs in grip, for example, is due to exaltation of virulence on the part of organisms common in the intestines and to their localization at certain points where the resistance has momentarily been diminished owing to exaggerated parasitism or to any other cause (multiple digestive insufficiencies, general diminution of the resistance of the patient by an intercurrent infection, etc.)."

M. S. MARSHALL.

SILENT TYPHUS FEVER IN MAN. J. TROISIER, R. CATTAN and Mlle. SIFFERLEN, *Ann. Inst. Pasteur* 47:492, 1931.

The question of the specific etiology of the exanthematic fever occurring around the Mediterranean basin was approached experimentally. Dog ticks, *Rhipicephalus*

sanguineus, the demonstrated vectors, were collected, ground and injected into a man, in whom an infection inapparente, as described by Nicolle, was induced. The Weil-Felix reaction was positive, but no systemic or local disturbance appeared. Transfer to a second subject resulted in similar observations. The blood of the first patient, injected into a monkey, induced a typical fever, a discrete exanthem and a Weil-Felix reaction. The blood of the monkey induced fever in guinea-pigs. The virus of the blood of the first human subject was also apparently passed through a guinea-pig to a monkey to a human subject, and through a series of three guinea-pigs to a monkey. Histologic study confirmed these observations (thrombophlebitis and perivascular infiltration). This virus of man, monkeys and guinea-pigs transmitted by the dog tick appears to be closely allied with typhus fever.

M. S. MARSHALL.

LIPASE CONTENT OF THE SERUM OF TUBERCULOUS CHILDREN. A. GRADNAUER, Beitr. z. Klin. d. Tuberk. **77**:725, 1931.

The lipase content was determined by the stalagmometric method of Rona and Michaelis, modified by Willstätter and Memmen. The serum of healthy children and adults shows, on the average, about the same amount of lipase, although large individual variations occur; that of patients with prognostically unfavorable pulmonary tuberculosis shows the level of lipase to be very low. A correlation between the lipase content and the general condition, the extent of the pulmonary involvement and the anatomic character of the pulmonary lesion is not demonstrable. Very low lipase values in pulmonary tuberculosis indicate a hopeless prognosis, but the same is not true in other organic involvement.

MAX PINNER.

FILTRABLE FORMS OF THE TUBERCLE BACILLUS. D. SZÜLE, Beitr. z. Klin. d. Tuberk. **78**:18, 1931.

Filtrates of tuberculous organs and of pure cultures were inoculated in a variety of mediums. In no case was growth obtained. Inoculation of animals with the same filtrates yielded negative results.

MAX PINNER.

TUBERCULOSIS OF THE BRONCHIAL NODES AS A CAUSE OF SEVERE HEMOPTYSIS IN OLD PEOPLE. A. ARNSTEIN, Beitr. z. Klin. d. Tuberk. **78**:55, 1931.

Hemoptysis caused by perforation of a tuberculous or anthracotic lymph node simultaneously into the bronchial tree and into a blood vessel is not a rare occurrence in old people. Ten cases are reported in some detail.

MAX PINNER.

SERUM CHOLESTEROL IN HIGH ALTITUDES AND ITS RELATION TO TUBERCULOSIS. A. BEHRMANN, Beitr. z. Klin. d. Tuberk. **78**:214, 1931.

Studies on twelve normal persons and on forty-seven tuberculous patients failed to show a definite influence of high altitude on the serum cholesterol. With productive lesions, the cholesterol content is normal or increased. In patients with progressive involvement and particularly in those who are cachectic, sub-normal values are frequently found.

MAX PINNER.

THE MAIN FORMS OF TUBERCULOSIS IN AUTOPSY MATERIAL. H. RÜDEL, Beitr. z. Klin. d. Tuberk. **78**:243, 1931.

This paper is based on the observations in the Pathologic Institute at Heidelberg from 1911 to 1930. During this time, a total of 14,967 autopsies were made. In 1,816 cases, making 12.1 per cent of the total, the main diagnosis was tuberculosis. Death from tuberculosis occurs with the highest frequency in the first

year of life, during puberty and in the third decennium. Generalizing forms of tuberculosis comprised 47 per cent of all cases of tuberculosis, and miliary forms constituted 42 per cent of all generalized forms. Of 856 cases of generalized tuberculosis, 73 were cases of acute miliary tuberculosis.

MAX PINNER.

TUBERCULOUS ENDAORTITIS. J. HAAS, Beitr. z. Klin. d. Tuberk. **78**:315, 1931.

In a man 58 years old who had died of subacute miliary tuberculosis a polypoid mass, the size of a bean, was found on the intima of the arcus of the aorta. This proved to be a slightly encapsulated caseous tuberculous mass containing large numbers of tubercle bacilli. Since caseous material easily oozed from this mass, it is assumed that it was the feeding focus for the miliary propagation. This focus, according to histologic studies, must have started in the intimal layer. No tuberculous foci were found in the adventitial layer.

MAX PINNER.

THE BIOLOGIC PROPERTIES OF SEVENTY-EIGHT STRAINS OF TUBERCLE BACILLI ISOLATED FROM INFANTS IN LÜBECK. H. J. TIEDEMANN and A. HÜBENER, Beitr. z. Klin. d. Tuberk. **78**:520, 1931.

The strains were obtained both from clinical material and from autopsy material from infants who had been vaccinated in Lübeck, supposedly with BCG. Only two of the strains were not pathogenic for guinea-pigs; these behaved in the same manner as does an undoubted BCG strain. One of these avirulent strains was obtained from autopsy material from a child who had died of a nontuberculous infection; the only tuberculous lesions found were small caseous foci in the mesenteric lymph nodes. The second avirulent strain was obtained from the gastric content of a child who completely recovered from an undiagnosed disease. All other strains were virulent for guinea-pigs, but not for rabbits. Their virulence for guinea-pigs was rather slight, but quite uniform, although they were derived from children with progressive, fatal and healing forms of the disease. Since even the strains from children who had died from acutely progressive tuberculosis showed low virulence for guinea-pigs, it must be concluded that virulence for guinea-pigs is in no way parallel to that for man. The strain "Kiel" which was present in the laboratory in Lübeck at the time of vaccination, and which is an atypical human strain, shows approximately the same degree of virulence for guinea-pigs as do the strains isolated from infants.

MAX PINNER.

THE PRODUCTION OF A GROWTH REGULATOR BY ASPERGILLUS NIGER. P. BOYSEN-JENSEN, Biochem. Ztschr. **239**:243, 1931.

Peptic digestion of fibrin, hemoglobin and casein does not result in production of a growth regulator. *Aspergillus niger* cultured in solutions of peptone or hemoglobin produces a growth regulator in considerable amounts. As the formation of this growth regulator occurs also in liquid mediums, it is easy to obtain large amounts of it. The unit of the growth regulator is that amount which dissolved in 100 cc. of water plus agar causes a difference of 1 cm. in the distance from the convex to the concave side of the avenacoleoptile.

WILHELM C. HUEPER.

HYPERERGIC SKIN INFLAMMATION IN THE HOG. K. NIEBERLE, Verhandl. d. deutsch. path. Gesellsch. **26**:239, 1931.

While the septic erysipelas of the hog is characterized by confluent erythema affecting the skin of the abdomen and thighs, the so-called chronic erysipelas shows diffuse necrosis of the skin on the back and breast. In the necrotic areas, *Bacillus rhusiopathiae suis* can be demonstrated. The second form of the disease occurs as a rule in hogs that are immunized several months previously against

erysipelas. Anatomically, a hyaline thrombosis of almost every blood vessel in the diseased skin is the most striking feature. The cutaneous necrosis represents an allergic reaction due to the combined action of antigen and antibody.

C. ALEXANDER HELLWIG.

POSTMORTEM OBSERVATIONS ON INFANTS WHO DIED IN LÜBECK AFTER IMMUNIZATION AGAINST TUBERCULOSIS. P. SCHUERMANN, *Verhandl. d. deutsch. path. Gesellsch.* **26**:265, 1931.

The anatomic examination of fifty infants post mortem revealed that as a result of oral infection primary tuberculous lesions may be present in any organ that can be reached from the mouth. Only in eleven infants was the primary lesion found in a single organ, the small intestine, while in all the others primary lesions were present at the same time in two or more organs (small intestine, region of the neck, middle portion of the digestive tract, lung). Except in the small intestine, the primary lesion was found most frequently in the tonsils and in the middle ear. As infections in unusual locations were observed: primary tuberculosis of the duodenum, stomach and esophagus and apparently secondary tuberculosis of the larynx and of a dental follicle. The duration of the disease in the fifty infants varied between nine and thirty-four weeks. The causes of death were meningitis, inanition, intestinal obstruction and some rarer complications. Not infrequently, a nonspecific acute or subacute interstitial hepatitis was observed.

C. ALEXANDER HELLWIG.

THE RELATION OF THE NUMBER OF TUBERCLE BACILLI TO THE LESION. A. SCHMINCKE and SANTO, *Verhandl. d. deutsch. path. Gesellsch.* **26**:275, 1931.

Intracorneal and subcutaneous injections of different amounts of tubercle bacilli were given to rabbits, and it was found that the severity of the primary lesion ran parallel to the number of injected organisms. The secondary lesions, however, in the regional lymph glands, were without relation to the number of infecting tubercle bacilli, but were always maximal reactions. Systematic histologic and bacteriologic examinations of tuberculous lesions in human lungs showed that the severity of the tuberculous process does not depend on the quantity of the infecting organisms. In acute forms of pulmonary tuberculosis small amounts of organisms may be found, and, on the other hand, large numbers of tubercle bacilli may be present in chronic lesions. The deciding factor in the morphology of the tuberculous process is apparently the individual resistance against tubercle bacilli, whether congenital or required.

C. ALEXANDER HELLWIG.

## Tumors

AN EMBRYONIC TUMOUR OF THE KIDNEY IN FOETUS. G. W. NICHOLSON, *J. Path. & Bact.* **34**:711, 1931.

This is an account of a single case, with a comparison with physiologic development. I use the name "embryonic tumor" instead of "mixed tumor" or "adenosarcoma," as it accentuates the most characteristic morphologic feature of the tumor: It mimics or, as I try to show, retains the structure (and functions) of the developing kidney with a surprising degree of accuracy, in general outline and in detail. It is an interesting fact, used to the present day as an argument for the "congenital" origin of tumors in general, that the specimen, like others of its kind, was present at birth. It is therefore congenital and embryonic in every sense. And it is true that by far the greater majority of these tumors are observed in the first years of life. But birth is a mere incident in the life of the individual. It introduces him to a new environment to which he must adapt himself: his physiologic reactions are modified and evoked, but no new reactions are acquired. Does not this argument apply with equal force to his reactions to

an abnormal, or accidental, environment inside and outside the uterus? I see these embryonic tumors of the kidneys having their chief importance for oncology, not as congenital new growths, but as developmental malformations: they represent the physiologic evolution of a somatic tissue after abnormal stimulation at a time when growth is active and differentiation begins. They differ from the developing organ in that growth continues, and differentiation is aborted. The tumor, as I conceive it, does not arise in a malformed organ, but represents—and is—the malformed organ. Paul (1886) very rightly observed that “a normal tissue is just as congenital as a rudiment” or cell rest. Every tumor—like every physiologic organ—is truly congenital and truly acquired, whether it arises in an embryo as a reaction to an unknown stimulus or in a centenarian as a reaction to x-rays, soot, tar or what not. For the reaction is innate in either case, and therefore congenital and entirely physiologic; the stimulus alone is extraneous to the reacting part, acquired and pathologic. This is the plain teaching of pathology—free from theory—as I understand it.

AUTHOR'S SUMMARY.

THE ANTI-CARCINOGENIC ACTION OF DICHLORODIETHYLSULPHIDE (MUSTARD GAS). I. BERENBLUM, J. Path. & Bact. **34**:731, 1931.

The inhibition of the induction of warts that results when mustard gas is added to a carcinogenic tar is due to some local action of the mustard gas on the tissues, causing the latter to become refractory to the carcinogenic action of the tar. This refractory state develops almost as soon as the mustard gas treatment is begun, and subsides soon after this treatment is discontinued. While the induction of a wart is inhibited, neither the preliminary hyperplasia of the epithelium nor the subsequent growth of the wart is in any way interfered with, when mustard gas is allowed to act on the skin. In brief, therefore, the inhibitory effect is a process, strictly limited in extent and time of action, which interferes with the induction of a wart without influencing the epithelial changes that either precede or follow the actual induction of the wart. Though it has been possible to produce a simple wart in one mouse as a result of repeated application of a 0.05 per cent solution of mustard gas in liquid paraffin, it is doubtful whether mustard gas can be safely considered as a carcinogenic agent. While the earlier experiments were in keeping with the view that the inhibition was due merely to the production of a superoptimal degree of irritation, subsequent experiments have failed to support this hypothesis. It becomes necessary, therefore, to consider whether the inhibition is after all due to some specific chemical action of mustard gas on the tissues. The fact that mustard gas can inhibit the induction of tar warts without interfering with the early epithelial hyperplasia suggests that no parallel can be drawn between the amount of hyperplasia produced by an irritant and the carcinogenicity of that irritant.

AUTHOR'S SUMMARY.

RETICULUM CELL CARCINOMA OF THE THYMUS. S. McDONALD, JR., J. Path. & Bact. **35**:1, 1932.

A locally malignant reticulum cell carcinoma of the thymus gland in a man, aged 59, is described. It is suggested that the neoplasm originated in a portion of involuted thymic tissue.

AUTHOR'S SUMMARY.

MITOSIS IN THE HEPATIC METASTASES OF MALIGNANT TUMORS. R. A. WILLIS, J. Path. & Bact. **35**:11, 1932.

A simple and reliable technic for the postmortem enumeration of the proportion of the cells in mitosis in malignant tumors is described. Nine tumors of various nature and origin and their hepatic metastases have been examined. In all, the mitotic activity of the hepatic deposits decidedly exceed that of the primary growths. Evidence is advanced in support of the view that hepatic tissue is a

highly favorable medium for the growth of most kinds of malignant cells, and it is suggested that this may be related to the high carbohydrate content and low arterial vascularity of the liver.

AUTHOR'S SUMMARY.

PLASMACYTOMA OF THE NASOPHARYNX. J. W. S. BLACKLOCK and C. MACARTNEY, *J. Path. & Bact.* **35**:69, 1932.

The case described is one of multiple small, plasma cell tumors occurring in the nasopharynx of a man, aged 64.

AUTHORS' SUMMARY.

PRIMARY CARCINOMA OF THE LUNG. E. PEKELIS, *Tumori* **5**:33, 1931.

This article compares the statistics of the various institutes of pathology in Italy and in foreign countries, describes the gross and microscopic picture of pulmonary carcinoma, and discusses the possible etiologic factors in the production of the disease. Five cases of pulmonary carcinoma are described. Three of these were bronchial adenocarcinoma, one was a diffuse infiltrative carcinoma, and one was a papillomatous carcinoma. In all of the cases, the new growth originated in the hilus, and macroscopically was of a nodular type.

G. PATRASSI.

THE SOLUBILITY OF CARCINOMA LIPOID IN SERUM. R. WILLHEIM and K. STERN, *Biochem. Ztschr.* **239**:473, 1931.

The authors attempt to substitute for the unreliable method of cell counts in the test for carcinolysis (Freund and Kaminer) the measurement of the solubility of carcinoma lipoids. They found that normal serum can dissolve carcinoma lipoids. The amounts of lipoid dissolved increase with increasing amounts of normal serum. Carcinoma serum, on the other hand, gives off its own lipoids to the carcinoma lipoids used. The lipoids were extracted from carcinoma cells freed from adherent connective tissue and detritus. They are unstable and show marked differences in sensitivity in regard to the reaction described. Fractioning of the lipoids did not give better results.

WILHELM C. HUEPER.

EXPERIMENTAL CHANGES IN THE SERUM REACTION TOWARD CARCINOMA CELLS. R. WILLHEIM and K. STERN, *Biochem. Ztschr.* **239**:484, 1931.

Human carcinoma cells were injected intraperitoneally and solutions of carcinoma lipoids intravenously into rabbits. They showed then the same curves of lipid solubility as carriers of human carcinoma. Control examinations with normal cells and lipoids yielded negative results. The immunologic aspect of these findings in regard to susceptibility to carcinoma is discussed.

WILHELM C. HUEPER.

MALIGNANT RHABDOMYOMA OF THE PROSTATE GLAND IN A CHILD. K. KATZMANN, *Frankfurt. Ztschr. f. Path.* **41**:297, 1931.

Such a tumor is reported in a 13 months old child. Grossly, it was of the size of a hazelnut and of very firm consistency, and protruded into the urinary bladder. It had led to a compression of the mouth of the urethra. There also was found a cystopyelitis. Histologically, the tumor consisted of transversely striated muscle fibers, with many cells. The cells had many nuclei, were rich in chromatin and showed several nucleoli. In addition to the transverse striations, a longitudinal striation of the fibers was occasionally observed. An invasion of the blood vessels by the muscle elements was often found. Thirteen cases of rhabdomyoma of the prostate gland reported in the literature are also briefly discussed.

O. SAPHIR.

THE BLOOD OF PATIENTS WITH CANCER. G. CREUZBERG, F. DANNMEYER, O. HARTLEB, E. L. LEDERER, L. VON NOEL, J. SCHUBERT, H. SEEL and L. TREPLIN, *Strahlentherapie* **42**:609, 1931.

Von Noel, from studies made on the ether-soluble (and presumably lipid) extracts of blood serums of cancerous and noncancerous persons, shows differences between the two. In cancer, the fatty acids of the blood are preponderately of low molecular weight. There are also differences in the values of saponification, free fatty acid, neutral fats and unsaponifiable material. These data are utilized by von Noel in the construction of a cancer number (Krebszahl), its magnitude being indicative of the presence or the absence of cancer.

Dannmeyer, Hartleb and Schubert call attention to the differences in the absorption spectra from cancerous and noncancerous persons. They consider these differences sufficiently constant to be of diagnostic value.

Lederer measured the ability of various samples of blood serum to inhibit the sedimentation of colloiddally dispersed Berlin blue. Serum from noncancerous persons, would, in greater dilution, exert a protective colloid action on this dye, than would the serum from the blood of cancerous persons. The investigator considers this as of some diagnostic importance.

Creuzberg and Seelin tested the therapeutic effects of injections of (1) extracts of cancerous serum, prepared by von Noel, (2) extracts of noncancerous serum and (3) furosaccharate (? probably furfural or methyl furfural) in conjunction with ultraviolet irradiation on tumor-bearing mice. The first were without effect, but the second and third inhibited the growth of the tumors.

Treplin considers the cancer number of von Noel and the spectroscopic picture of Dannmeyer and Hartleb as of more diagnostic significance than the Lederer sedimentation test.

### Medicolegal Pathology

INCIDENCE AND SITUATION OF MYOCARDIAL INFARCTION. A. R. BARNES and R. G. BALL, *Am. J. M. Sc.* **183**:215, 1932.

One thousand consecutive, nonselected postmortem examinations are reviewed. In forty-nine cases (4.9 per cent) the examination showed myocardial infarction. In twelve of these the infarcts were multiple. The left anterior descending branch of the left coronary artery was involved in twenty-eight cases; the circumflex branch of the left coronary artery, in seventeen cases, and the right coronary artery, in twenty cases.

WILLIAM FREEMAN.

OCCUPATIONAL MELANOSIS. L. M. WIEDER, *Arch. Dermat. & Syph.* **25**:624, 1932.

Wieder reviews the literature and presents the case of a 37 year old white chemist who handled finished dyes, naphthalene acids and vat dye intermediates. Within four years a dermatitis developed, in which melanosis of the face and neck was the most prominent symptom. Biopsy of the lesion revealed intracellular edema of the epidermis and moderate follicular hyperkeratosis with considerable pigmentary activity in the basal layer of cells. Melanoblasts were abundant along the basal layer, and chromatophores with much pigment were numerous in the superficial dermis. Vacuolization of the melanoblasts produced a pseudo-edematous appearance at the dermo-epidermal junction. There was also moderate pigmentation in the malpighian layer. No vascular or connective tissue changes were found.

WILLIAM FREEMAN.

HYDROCYANIC ACID GAS POISONING BY ABSORPTION THROUGH THE SKIN. P. DRINKER, *J. Indust. Hyg.* **14**:1, 1932.

Drinker reviews the literature indicating that hydrocyanic acid gas may be absorbed through the skin of animals and human beings, thereby leading to poisoning and possibly death.

WILLIAM FREEMAN.

SUDDEN DEATH FOLLOWING CISTERNAL PUNCTURE. B. C. RUSSUM and M. W. BARRY, Nebraska State M. J. **17:30**, 1932.

The authors review the literature and report two cases in which death followed cisternal puncture. In both instances (involving 4 months old girls) death was caused by hemorrhage brought on by the puncture. In both instances, the pial vessels were punctured.

WILLIAM FREEMAN.

SPONTANEOUS RUPTURE OF THE HEART. F. R. BARNES, New England J. Med. **206:631**, 1932.

A 50 year old white man suddenly collapsed and died, receiving a laceration of his forehead in hitting a stove. He had previously complained of severe pain in the left arm and chest. Death was instantaneous. Postmortem examination revealed a perforating laceration of the left ventricular myocardium, 1 inch (2.5 cm.) upward from the apex. The literature of such a condition is reviewed from a statistical basis.

WILLIAM FREEMAN.

A CASE OF ACUTE YELLOW ATROPHY OF THE LIVER DUE TO CINCHOPHEN. S. C. LIND, Ohio State M. J. **28:28**, 1932.

A case of acute yellow atrophy of the liver due to ingestion of 90 grains (5.8 Gm.) of cinchophen is recorded. The patient lived two and one-half months following the administration of the drug.

WILLIAM FREEMAN.

TRAUMATIC APPENDICITIS. L. MILSON, Wisconsin M. J. **31:100**, 1932.

Milson reports the case of a man, 19 years of age, in excellent health, who was struck in the abdomen by an automobile traveling at a fairly rapid rate of speed. A sharp abdominal pain with nausea set in immediately after the injury. All of the signs and symptoms of acute appendicitis developed. Operation revealed an acutely inflamed appendix with a large fecalith obstructing the lumen and protruding through the appendiceal mucosa into, but not through, the submucosa. No other abdominal viscera were apparently injured. Milson believes that severe injuries to the abdomen may play a part in the etiology of acute appendicitis.

WILLIAM FREEMAN.

SUDDEN DEATH FROM SHOCK IN ATTEMPTED ABORTION. M. DUFOUR, Ann. de méd. lég. **12:7**, 1932.

A girl, 16 years of age, had a cannula introduced into the vagina so that water could be forced into the uterine cavity. The patient died soon after the cannula was inserted. All of the organs were found normal at autopsy except for excoriations and erosions of the cervix. The embryo was intact. The conclusion by the author is that sudden death was due to shock during an attempted abortion.

H. S. THATCHER.

EXHUMATION OF NEW-BORN INFANTS. G. STRASSMANN, Beitr. z. gerichtl. Med. **11:36**, 1931.

Strassmann believes that autopsies on exhumed new-born infants may occasionally reveal whether or not the infant lived and whether it died from birth trauma or from natural causes. The Gram-Weigert and sudan stains are especially valuable in demonstrating inhaled amniotic fluid.

O. SAPHIR.



IMPORTANCE OF MEDICOLEGAL EXPERT AT THE SCENE OF CRIME. ZANGGER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:101, 1931.

In the realm of legal medicine belongs not only the study of the medical phases encountered, but also, and mainly, the logical correlation of all the facts present on the premises of crime or accident. The action of a force or violence that caused injury to or destruction of the human body, and also characteristic damages to the surrounding objects, must be accurately analyzed and their mutual relations considered and evaluated with the accompanying circumstances, in order to arrive at and to express a scientifically correct opinion of all the happenings. A biologiomedically and medicolegally trained observer, with his background of sound scientific knowledge can render valuable service in gathering conclusive evidence at the scene of crime. In a trial, the testimony of lay witnesses as to their observations may be inaccurate and misleading, and conclusions arrived at from their findings may be erroneous and deceiving, but the expertly gathered, scientifically analyzed and convincingly introduced proof of facts will clear up the apparent inconsistencies and discrepancies and lead to justice.

E. L. MILOSLAVICH.

THE MEDICOLEGAL AUTOPSY FROM A CRIMINALISTIC STANDPOINT. NIPPE, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:103, 1931.

The purpose of the medicolegal autopsy is not only to detect and register the pathologic changes, damages and injuries present and to establish the cause of death, but to determine a likely fault, guilt or crime. From the anatomic character of a wound, one can occasionally conclude what type of weapon was used. Presence of foreign bodies in a wound, for instance, minute particles or parts of an instrument, such as fragments of a knife, razor, glass splinters from a bottle or jar, etc., may serve to identify the weapon and its owner. The larger fragments are easily removed from the bed of the wound or the canal with forceps, or the entire wounded area may be excised, minutely washed and shaken, and the sediment centrifugated. Unforeseen, important findings may be obtained. Examination of urine for albumin is of certain practical value only when one critically considers the microscopic picture of the kidneys, since cadaveric desquamation of the epithelial elements of the bladder and autolytic processes may lead one to incorrect conclusions as to the presence of albumin. Sugar may be unexpectedly detected in the urine of persons who died following acute lesions of the brain, as hemorrhages, while a previous clinical examination, prior to the injury to the brain, was negative. The urinary bladder rarely shows postmortem contraction. The uterus exhibits a similar behavior following miscarriage or delivery. The uterus, for instance, presents a peculiar resistance to putrefactive processes. The rigor mortis of the heart and the fragmentation of the myocardial fibers are quite reliable signs of the functional power and activity of the heart. In exhumed, unembalmed bodies, the intestinal canal is usually found better preserved than the parenchymatous organs. Later on after death the consistency of the contents of the large intestines may change; one may observe either liquefaction or inspissation of the fecal material. In examining the hair, one may detect important findings as to cut surface—changes due to burns, injuries by blunt force, by instruments, by automobile impact or by electric currents, gunshot effects, etc. The cadaveric hypostasis, even in cases of lethal hemorrhages, may appear after one-half hour or three quarters of an hour, and up to four hours after death the change in the position of the body will result in a change in the location of the hypostatic areas. The cadaveric rigidity starts, as a rule, three quarters of an hour after death, in the jaw, and after from one and one-half hours to two hours it is well pronounced in the extremities. After it disappears it may spontaneously reappear, and a revolver placed in the hand of a dead person may later be found grasped firmly by the rigid hand. This phenomenon may lead to misinterpretation with respect to suicide. About twelve hours after death the rigidity is complete, if the body has been in a cool environment. If the rigor mortis was artificially loosened, it

does not recur; otherwise, it disappears on account of autolytic processes or decomposition. Should the body be kept in a cool place, the rigor mortis may persist for many weeks and directly pass into desiccation and mummification of the corpse. In cases of gunshot wounds, one should never omit an accurate inspection of the clothing. If a pistol is discharged at a very close range, the powder gases may expand between the surface of the body and the clothing, for example the shirt, and produce a smudging of the skin and of the inner aspect of the shirt. Advancing putrefaction may obscure the characteristics of a shot fired at a close distance, but application of hydrogen peroxide solution may bring out the smudged areas. Microscopic examination of the canal of the wound may disclose presence of torn particles or fibers of fabric. E. L. MILOSLAVICH.

SUDDEN DEATH FROM NATURAL CAUSES IN ADULTS. GUNTHER WEYRICH, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 18:211, 1931.

A statistical analysis of 2,668 cases of sudden death discloses that in nearly half (42 per cent) the unexpected death occurred on account of disease of the circulatory apparatus, 550 cases showing coronary sclerosis, with predominance of males. Considering the males alone, in 47 per cent death resulted from cardiac or aortic diseases. The mortality was highest in the sixth decade of age. Of 623 instances of sudden death from diseases of the respiratory organs, 267 resulted from pneumonia and pleurisy, while pulmonary embolism was noted in 47 autopsies. The highest mortality was observed during the month of February, the lowest in August. While every fifth case of sudden death was due to coronary sclerosis, cerebral apoplexy was found in only every twentieth instance. This statistical fact is of great practical importance, since, in a case of unexpected sudden death, the average physician ordinarily assumes a hemorrhage of the brain. Three times death from paralysis of the heart occurred during sexual intercourse. Considering the relation between occupation and sudden death due to cardiac disease, this study reveals that mental workers are represented by an unusually high percentage as compared with persons engaged in heavy manual labor.

E. L. MILOSLAVICH.

PATHOLOGY OF SUDDEN DEATH FROM NATURAL CAUSES DURING COITUS. G. SCHRADER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 18:223, 1931.

In legal medicine, sudden death forms an important chapter, since often dubious circumstances surround the unexpected catastrophe. This is particularly true in instances of lethal collapse of an apparently healthy person during the sexual act. Three such cases are presented, each of peculiar criminalistic significance. The first case involved a man, 50 years of age, visiting a prostitute. The man was found moribund in her room, while she was apparently unconscious but rapidly recovered. A gas jet was open, and the odor of gas was noticeable. The autopsy disclosed syphilitic mesaortitis with thrombotic deposits and alcoholic intoxication, but no evidence of carbon monoxide poisoning. The prostitute, frightened by the sudden collapse of her companion, had tried to feign an accident. In the second case, a young sportsman died suddenly during sexual intercourse. The postmortem examination disclosed a large amount of blood in the subarachnoid space at the base of the brain, due to a hemorrhage from the posterior part of the basilar artery, which showed an old tear with an intramural hematoma. The latter lesion was explained as the result of an indirect trauma to the head sustained eight days prior to death owing to a fall while exercising. In the third case, a 27 year old woman suddenly expired during the first night of her honeymoon. At autopsy, syphilitic mesaortitis and stenosis of the coronary ostia were found. Thrombotic material was attached to the diseased ascending aorta and extended into the opening of the left coronary artery.

E. L. MILOSLAVICH.

ASPIRATION OF PARTICLES OF BRAIN INTO THE RESPIRATORY TRACT IN INJURY TO THE SKULL. D. HIDASSY, Orvosi hetil. 1:92, 1931.

A boy, 9 years old, received a fatal fracture of the base of the skull from being run over by the wheel of a truck. At the autopsy there was found a considerable amount of brain tissue in the larynx and bronchi in addition to fluid blood.

### Technical

IMMEDIATE AND DIRECT METHODS OF TYPING PNEUMOCOCCI. R. R. ARMSTRONG, Brit. M. J. 1:187, 1932, and W. R. LOGAN and J. T. SMEALL, *ibid.* 1:189, 1932.

The procedure used by Armstrong is as follows: "A suitable fleck of sputum is selected. Three small samples of this are placed, equidistant, on a microscope slide, and numbered 1, 2 and 3. Each sample is emulsified with four times its volume of the corresponding diagnostic serum, the addition of serum and emulsification being conveniently performed with a platinum loop. Cover glasses are applied, and the slide is set aside for a few minutes while a further sample from the selected fleck of sputum is smeared on a slide, fixed by heat, and stained by Gram's method. The general bacterial flora of the sputum and the number of pneumococci present are apparent at a glance in the stained film, which exactly represents the characters of the sputum samples selected for the diagnostic test. It is of special value to be informed in advance of these characters, for if the pneumococci are plentiful, a positive result in the typing will be apparent at once; no time need be wasted in useless search, therefore, when, as in the case of a group IV infection, there is no reaction. The slide carrying the fresh emulsions of sputum and specific serums is now examined by means of a 4 ocular, one-sixth objective, and plane mirror, the condenser being removed. Whereas the unstained pneumococci, when present in small numbers, are but just visible in the case of a negative reaction, the result in the case of a positive reaction is a conspicuous increase in the size of the individual pneumococcus, due to conjugation of coccus and homologous antibody. The enlarged cocci have a characteristic ground-glass appearance, with a highly refractive peripheral zone. A positive reaction is at times appreciable to the naked eye on holding the preparation to the light. The positive, as compared with its companions on the same slide (which serve as controls), is seen to be opalescent, owing to the great increase in size of the "sensitized" cocci. When the pneumococci are thickly coated with seromucinous pneumonic secretion, the characteristic appearances develop more slowly as the specific serum soaks its way through. In such cases twenty minutes may elapse before full completion, although type may be distinguished much sooner by the change in those pairs that are floating free."

The technic used by Logan and Smeall for the direct typing of pneumococci is practically that described by Armstrong. An emulsion of the sputum is made with physiologic solution of sodium chloride. Four thin glass slides are marked I, II, III and control, and a large loopful of the undiluted type serum is placed on the appropriate slide, a drop of physiologic solution of sodium chloride being put on the control slide. The sputum emulsion is then taken up in a capillary pipet with a teat. A drop of the required size is placed beside each drop of serum and then mixed by tilting the slide backward and forward. Sometimes it is better to drop the emulsion on to the serum; in other cases, in which the consistency is more gelatinous, it is necessary to mix with a loop. A thin cover glass is placed on the mixture, which is then examined under the microscope. The authors use a one-twelfth oil-immersion lens, with the substage condenser racked down a little and the diaphragm closed to an extent that has to be constantly varied to get the best results. The plane mirror and a bright artificial light are employed. In cases in which pneumococci are present in large numbers, the swelling of the individual pneumococcus and the appearance of the dark line

sharply outlining the capsule, along with a darkening of the body of the pneumococcus itself — distinct from the whitish, sometimes almost greenish-white, capsular substance — are strikingly seen when the homologous serum has been used. In the other slides the pneumococci are seen to be much smaller, with a small halo, which is a little lighter in color than the surrounding sputum, but there is no dark line sharply defining the outline of the capsule. In specimens in which pneumococci are scanty it is sometimes only in the slide with the specific type-serum that they stand out and become recognizable, while, in specimens containing many streptococci in diplococcal form, again it is only the reaction to the specific serum that indicates which are pneumococci. With some specimens the test has to be performed several times before a successful result is obtained.

INTRAVENOUS INJECTION OF CONGO-RED IN THE DIAGNOSIS OF AMYLOID DISEASE. J. E. WALLACE, *Lancet* **1**:391, 1932.

Amyloid material absorbs congo-red. If an aqueous solution is injected intravenously, the decrease of the dye in the plasma or serum may be estimated colorimetrically; normally from 10 to 30 per cent disappears in one hour, but in amyloid disease the disappearance is much more rapid.

AUTOPSY TECHNIC FOR EXAMINING NASAL SINUSES AND THE CEREBELLUM. A. SCHMINCKE, *Centralbl. f. allg. Path. u. path. Anat.* **53**:273, 1932.

The skin incisions employed in this method are carried across the shoulders from the outermost ends of the usual infraclavicular cuts and meet in the midline between the shoulders. Two flaps are then formed by a cut upward from this junction to the nape of the neck. After freeing the skin from the subcutaneous tissues, the mandible is disarticulated, and by chiseling through the maxillae the sinuses can be exposed. Reflection of the skin from the neck forward and upward allows exposure of the cranium for the usual cut in the greatest horizontal diameter for removal of the brain. When it is desirable to remove the brain and spinal cord in one piece, the two additional saw-cuts are made through the base of the cranium to the edges of the foramen magnum. Schmincke advocates this method for the demonstration of tumors of the cerebellopontile angle. Restoration of the body is simplified if the skin sutures are begun at the angle between the shoulders.

GEORGE RUKSTINAT.

THE SEROLOGIC DIAGNOSIS OF SYPHILIS WITH THE CITOCHOL REACTION OF SACHS-WITERSKY. S. L. SCHIRWINDT and A. V. ALEKSEEVA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:54, 1931.

The reaction was compared with the Wassermann and Kahn reactions and with the lentochol-reaction of Sachs-Georgi. It was proved superior to the lentochol-reaction; it was somewhat less sensitive than the Wassermann and Kahn reactions in the early stages of syphilis, in mixed chancre and in congenital syphilis, but almost equally sensitive in all other forms of syphilis. A tendency toward nonspecific positive reactions was observed. The results with cerebrospinal fluid, which were unsatisfactory with the original method, improved after cholesterol was added to the antigen and improved still more after precipitation of the globulin according to the procedure of Kahn.

I. DAVIDSON.

BLOOD CULTURES OF TUBERCLE BACILLI ACCORDING TO LÖWENSTEIN'S METHOD. UNVERRICHT and S. DOSQUET, *Ztschr. f. Tuberk.* **63**:338, 1932, and N. B. OEKONOMOPOULO, B. PAPANIKOLAOU and G. JOANNIDES, *ibid.* **63**:340, 1932.

In both these articles essentially negative results are reported from cultivating the blood for tubercle bacilli according to the method described by Löwenstein.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, March 24, 1932*

PAUL KLEMPERER, *President, in the Chair*

### A CASE OF MALIGNANT CARCINOID OF THE ILEUM. LOUISE H. MEEKER.

The patient, 61 years old, was admitted to the hospital because of abdominal cramps. The past history showed attacks of cramps relieved by cathartics. The preoperative diagnosis was intestinal obstruction. At operation, kinking of the intestine with dilatation above was found, and resection of about 3 feet (91 cm.) of the lower part of the ileum was done.

The gross specimen was a portion of the small intestine, 550 mm. in length. The mesentery was contracted so as to produce sharp kinking of the bowel, and in the mesentery there were opaque, somewhat yellow thickenings. When the intestine was opened, the mucous membrane presented numerous hemorrhagic spots and at one place a large area of extravasated blood. Here the wall of the intestine was distended to form a pouch about 50 mm. in diameter. In the lumen there was considerable mucus stained by bile.

Microscopic sections through the firm portions in the mesentery at the point of kinking of the intestine showed a dense fibrous stroma, in which there were many irregular nests of deeply staining cells. These cells had slightly vesicular, round nuclei, uniform in size, and a small amount of cytoplasm. Some of them resembled lymphocytes. They were grouped in well defined nests, and within these nests the epithelial cells were occasionally arranged around a circular lumen, indicating a tendency to form a gland duct. Mitotic figures were not recognized. The cell nests were intimately associated with numerous large nerve trunks. The neoplastic epithelium extended beyond the intestinal wall into the mesentery and invaded several lymph nodes.

The final diagnosis was "malignant neurocarcinoma," based on (1) the chromaffin color of portions of the gross tumor; (2) the typical rosette formations, each about a cavity containing colloid-like material, and lack of mitotic figures; (3) the proliferation in relation to the nerves, and (4) the metastases of the tumor to the lymph nodes.

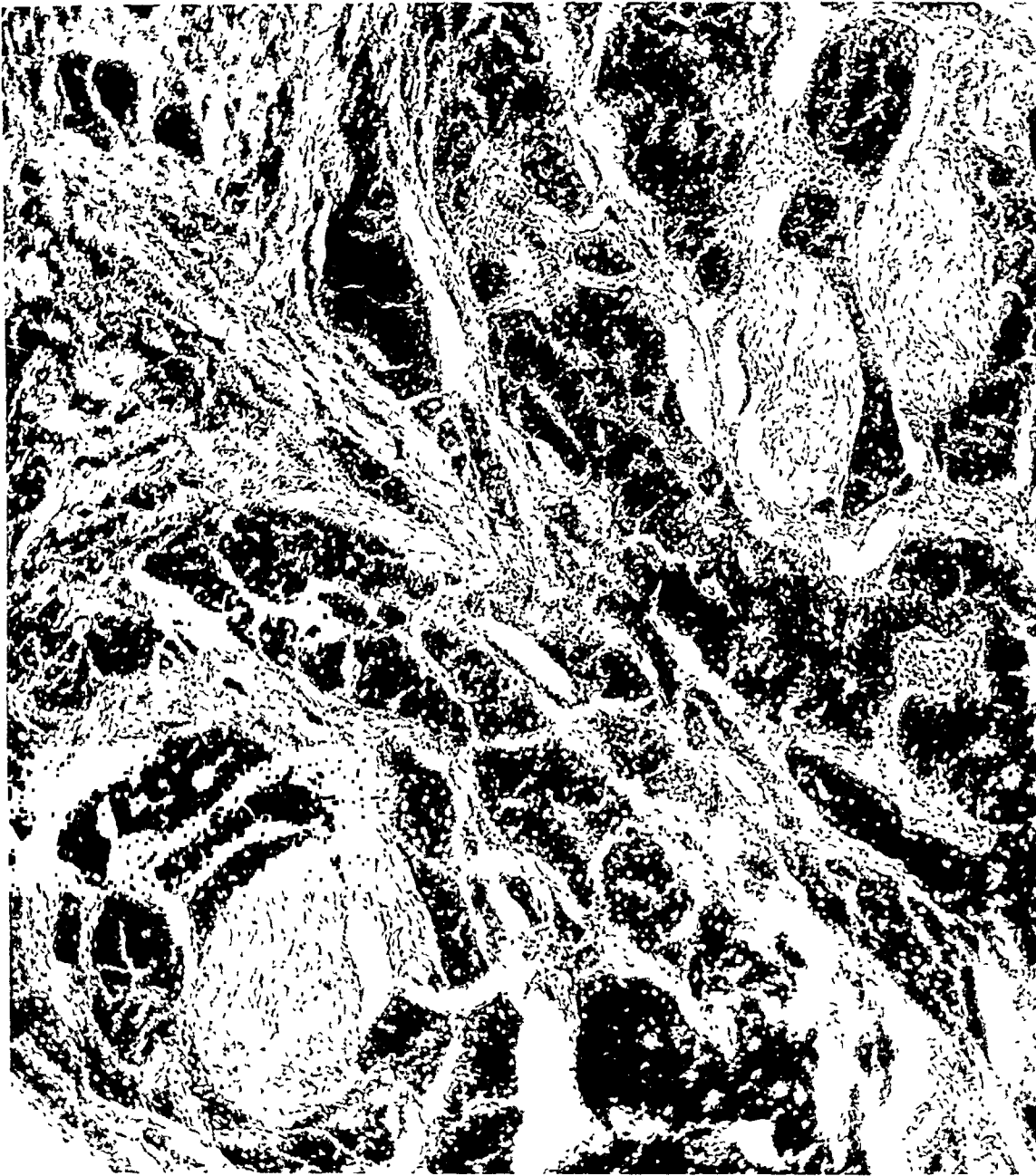
The lack of union with glands of the intestinal mucosa may be explained, according to Masson, by former epithelial buds sending isolated cells into the nerve tissue.

### ACTINOMYCOSIS OF THE BRONCHI AND LIVER. CHARLES T. OLCOTT.

A specimen of an abscess of the liver occurring in association with bronchiectasis of the lower part of the right lung was presented. Actinomycetes were found both in the lung and in a solitary abscess in the upper surface of the liver. Although there was a history of a mass in the cecal region two years before, no remnant of this was found at autopsy.

### ACTINOMYCOSIS OF THE MESENTERY OF THE COLON. CHARLES T. OLCOTT.

A lantern slide of a surgical specimen obtained by Dr. John H. Garlock was shown. A woman, aged 53, showed symptoms of obstruction in the transverse colon. Resection showed no lesions in the intestine itself, but actinomycetes were



Microscopic appearance of the carcinoid of the ileum in Dr. Meeker's case. The masses of tumor cells show small lumina. The light areas are nerve trunks.

found in a hemorrhagic area of the mesentery. Resection was followed by a cure of two and a half years' duration.

#### DISCUSSION

ALFRED PLAUT: In regard to the last case, may I make somewhat pessimistic remarks? I, too, once thought that I had seen a cure in a case of abdominal actinomycosis. I followed the patient for five and a half years, during which time she remained well, but I finally heard that she had retired to the parental farm and had died of an unknown disease. There was no autopsy, but it is reasonable to presume that she died of an actinomycotic process. I do not know of any permanent cure of actinomycosis. Actinomycosis is a much more frequent disease than we think. Many years ago a surgeon came to me about a case, and said that he felt sure it must be actinomycosis, but that he had gone to many pathologists and bacteriologists and had got no help. He asked if I could help him, and I said, "Probably not," but I finally found one granule, just as Dr. Olcott mentioned, and I am sure that if we had not had the time to make such tedious examinations, the one granule would have been missed, and the diagnosis with it.

#### AN UNUSUAL ADENOCARCINOMA OF THE STOMACH. EDWARD B. GREENSPAN.

A case of adenocarcinoma of the stomach in a 41 year old man was presented because of the remarkable resemblance of the tumor grossly and histologically to chorio-epithelioma.

The tumor grew from the posterior wall of the stomach near the pylorus, causing a large filling defect on the greater curvature. It was a mushroom-shaped, hemorrhagic tumor, measuring 5 by 5 by 2 cm. The regional lymph nodes revealed metastases. There were oval, hemorrhagic nodules in the liver, greatly resembling metastatic chorio-epithelioma. There was also carcinomatous lymphangitis of the pleura with nodules in the pleura and in the pulmonary parenchyma.

Microscopically, the remarkable resemblance of the metastatic nodules in the liver to chorio-epithelioma was noted. Large, multinucleated giant cells, suggestive of syncytial cells, and oval cells with pale, vesicular nuclei, resembling Langhans' cells, made up the tumor. Here and there were lakes of blood surrounded entirely by tumor tissue. A section through the tumor of the stomach revealed nests of cells showing frequent mitoses, arranged in the distinct pseudoglandular formation of adenocarcinoma, replacing a part of the mucosa. Here, as in the liver, one saw in the submucosa collections of cells resembling syncytial cells and Langhans' cells of the placenta, often surrounding collections of blood, and invading the muscular layers and at one point the muscularis.

In 1905, Davidsohn reported a case in which histologic examination of one portion of the tumor revealed great similarity to chorio-epithelioma, while in other portions definite carcinoma was seen. He believed that he was dealing with two separate tumors. In 1907, Helmholtz published a description of a tumor of the stomach, which he labeled "A Syncytiomatous Tumor of the Stomach," and which he suggested was the result of a misplaced "anlage."

#### ISOLATED NECROTIZING ARTERITIS AND SUBACUTE GLOMERULONEPHRITIS IN A CASE OF GONOCOCCAL ENDOCARDITIS. MILTON HELPERN and MAX TRUBEK.

In a case of subacute glomerulonephritis of ten weeks' duration, death occurred from uremia with pericarditis. The illness developed eight weeks after gonorrheal urethritis, complicated by gonococcic ophthalmia. At necropsy, right-sided subacute gonococcic endocarditis of the pulmonic valve and typical subacute glomerulonephritis were found.

In addition, isolated necrotizing inflammatory lesions of the small arteries were found in the choroid coat of the eye and in the testicle. These vascular lesions

exhibited many of the morphologic features of periarteritis nodosa and were considered as belonging to that group. The larger arteries of the body were not involved, and the distribution of the lesions was not extensive enough to produce clinical manifestations.

The renal lesion is interpreted as a severe reaction to the toxin produced by the gonococci growing on the pulmonic valve. The vascular lesions, which in this case were anatomically dissociated from the kidney, were also considered to be a specific reaction to the same toxin.

The findings strengthen the conception that these vascular lesions and those of periarteritis nodosa do not represent a specific disease, but rather a reaction of the blood vessels to any of a whole group of toxins.

#### DISCUSSION

MAX TRUBEK: Gruber maintains that periarteritis nodosa may be caused by any one of a number of infections, identical arterial lesions occurring in the previously sensitized vessels. The preceding infection need not be a severe one—coryza, suppurating wounds, furuncles, sore throat, rheumatic fever, erysipelas, syphilis, gonorrhea and other conditions. His surmise as to the gonococcus seems proved in our case. The relatively low virulence of the gonococcus—judging from the long duration of the infection—furnished conditions for sepsis lenta, which Siegmund considered of importance in his case of streptococcic endocarditis associated with periarteritis nodosa.

We believe that in our patient the general infection by gonococci with endocarditis existed when the patient was being treated for the gonococcic ophthalmitis.

The evidence for a specific infection as the cause of periarteritis nodosa has been more or less in dispute. The positive results of von Hann and of Harris and Friedrich have kept the question open. Arkin accepted the virus theory without further proof. Otani obtained negative results, repeating the experiments of von Hann and using tissue and blood from a typical case of the disease for his animal inoculations.

The glomerular disease in our case was present without any arterial lesion. Renal complications in periarteritis nodosa may be purely arterial, purely glomerular or both. In our case, the glomerular nephritis was caused by the gonococcic toxemia. The periarteritis nodosa was an isolated occurrence, not the systemic disease.

Lesions in the eye in this disease have been previously described. The ophthalmoscopic changes are usually recorded as albuminuric retinitis. In our case, the retinal picture showed papilledema and hemorrhage.

Muller and Christeller mention lesions in the choroid in their cases. Böck and Herrenschwand demonstrated lesions in the extra-ocular muscles, central artery of the retina and ciliary arteries, respectively. Goldstein and Wexler showed a case with choroid involvement not unlike that in our case.

Lesions of the testis have been described a number of times, the involvement occasionally producing infarction and necrosis when both sets of vessels are affected.

Isolated lesions are not unusual. At times, characteristic changes occurring in only a few organs have been described. This fact may depend on the variety and number of the sections selected.

Microscopic identification of the disease, previously unsuspected, has been a frequent occurrence.

ISADORE GOLDSTLIN: Dr. Wexler and I studied three cases of periarteritis nodosa. We saw two of the patients during life. The patient whose case was reported by us was not seen during life. In one case there was bilateral papilledema with periarteritis in the other organs, but there was no periarteritis in the eye. In the case of the other patient whom I saw during life, I made a diagnosis of the malignant phase of essential hypertension. This was incorrect, for the man died of periarteritis nodosa. In that case there was no periarteritic lesion in the eye, while such lesions were present in some of the other organs. The case



reported by us was that of a woman, aged 19. There were microscopic lesions in the organs. In the eye, the iris, ciliary body and retina were normal, but the choroid disclosed lesions similar to those shown tonight. In the arteries we found either organized blood clots or fibrin, with marked proliferation of the endothelial cells and hyperplasia of the subendothelial tissue, consisting mostly of polyhedral cells. I think we were the first to call attention to this type of cell. We also drew attention to the sharply defined and thin media. There was necrosis of the arterial wall with marked infiltration of the adventitia and periadventitial tissue by lymphocytic cells. The same type of cell was found in the other organs. Mention was also made of the giant chromatophores, which showed so nicely in the case presented.

I do not believe that there is a typical picture of periarteritis nodosa of the fundus. Most of the cases are of the hypertensive neuroretinitis type, but the lesion shown here this evening apparently gives no fundus picture. If anything, this lesion should appear as a white spot, but we didn't see it, although our patient was studied during life, but not by one of us; so the question is still open whether periarteritis nodosa has a definite fundus picture.

ALFRED PLAUT: I would like to ask whether the authors are correct in calling the interesting lesions just presented periarteritis nodosa. I think not. The large amount of necrotic or fibrinoid change in the walls of the vessels, together with the small amount of inflammatory cellular reaction surrounding them, constitutes such a difference from the ordinary picture of periarteritis nodosa that I would rather call it necrotizing arteritis, and would not include it in the picture of periarteritis nodosa. I feel that twenty or thirty years from now the chapters on diseases of the arteries in our textbooks will be written differently. I do not know why the pathology of the arteries has been neglected so much, except for the chapters on arteriosclerosis and syphilis. More and more observations on peculiar lesions in arteries, which cannot be classified well, are coming into the literature. I feel that this is an important contribution to this chapter, most of which will be rewritten in the future, and I should not be astonished if twenty years from now many lesions that were previously listed under periarteritis nodosa are reclassified.

#### SOME PROBLEMS IN MEDICAL MYCOLOGY. J. G. HOPKINS.

The study of mycotic infections presents many problems of interest to the pathologist and the mycologist, which may be grouped under the following headings:

*Problems of Identification.*—The imperfect descriptions frequently given of fungi found in human infections make the classification of many such parasites extremely uncertain. The difficulty is partly inherent in their rudimentary morphologic state, which seldom presents characteristic structures by which they may be identified. Moreover, their morphologic characteristics in culture often bear no resemblance to those noted in the lesions. They vary in appearance when grown on different mediums, and, as Emmons has observed, variations that seem to be permanent develop in pure line strains. A clearing up of the confusion in regard to them demands that they be studied both by botanic and by bacteriologic methods. In studies of strains of *Monilia* morphologic descriptions combined with the results of immunologic tests have done much to clear up the confusion in regard to classification.

*Problems in Etiology.*—Infection with some fungi, such as *Actinomyces* and *Sporothrix*, apparently results from inoculation with the organism from some source outside the body. A search for reservoirs of infection in animals, in plants and in the soil is necessary to explain the etiology of these diseases and to control their spread.

*Problems in Infectivity.*—Some fungi are pathogenic under experimental conditions, reproducing in the laboratory the picture of the spontaneous disease. Others, such as *Actinomyces bovis*, are practically nonvirulent when experimentally

inoculated, and a study of the conditions under which they may produce lesions is essential to an understanding of these infections.

*Problems in Sensitization.*—The pathogenicity of some of these parasites in spite of their lack of virulence is explained by the fact that their hosts become sensitized to them. Studies in sensitization have already thrown much light on eruptions of the skin associated with dermatophytosis. Similar sensitization reactions occur in association with infections by monilia. Asthma is in some cases due to sensitization to fungi in dust, and there is evidence to show that certain cases of eczema are due to similar allergic reactions.

#### CLASSIFICATION OF YEASTLIKE PARASITES. RHODA W. BENHAM.

A definite classification of the yeastlike parasites is not possible due to the lack of knowledge as to the complete life history of these forms. However, some definite criteria for recognizing the types isolated from the various lesions and a uniform terminology need to be established. The names, *Blastomyces*, *Cryptococcus*, *Oidium*, *Monilia*, *Saccharomyces* and *Torula* have been used indiscriminately for a number of different organisms. Yeastlike organisms are found in deep-seated lesions of the skin, systemic infections, infections of the nervous system and superficial cutaneous lesions. A brief description of the organisms as seen in the lesions and as cultured from the more important of these diseases follows:

*American Blastomycosis* (Gilchrist, 1894).—In the lesion, the organism appears as a round, thick-walled, budding cell; in culture, as a filamentous form producing a white, fluffy colony—*Blastomycoides dermatitidis*.

*Coccidioidal Granuloma* (Wernicke, 1892).—In the lesion, the parasite is a large, round, thick-walled cell filled with numerous bodies considered to be spores. No buds are seen. In culture, the appearances are similar—*Blastomycoides immitis*.

*Chromoblastomycosis* (Pedroso, 1913).—Round, budding cells are seen in the lesion. The culture shows as dark brown or greenish, woolly growth—*Phialophora verrucosa*.

*Lymphangitis Epizootica* (Rivolta and Micellone, 1883).—The organism in the tissue appears as a small, oval body. No budding is shown. The culture is mycelial, resembling a faviform type of trichophyton—*Cryptococcus farciminosus*.

*European Blastomycosis* (Busse and Buschke, 1893).—Thick-walled, round cells are seen in tissue. The cultural growth is pasty. No mycelium and no ascospores are shown, only buds—*Saccharomyces hominis*.

*Torula Meningitis* (Stoddard and Cutler, 1916).—The torula appears in the lesion as a round, thick-walled, budding cell. The culture is yellow and mucilaginous. The organism is a round, budding cell, showing no mycelium and no ascospores—*Torula histolytica*.

There is also a meningitis due to a monilia-like organism (Ball, 1930).

*Superficial Cutaneous Lesions*—These include *erosio interdigitalis* (Kauffman-Wolff, 1914), chronic paronychia (Kumer, 1921), *perlèche* (Finnerud, 1929) and generalized dermatitis (White, 1929). In the lesions, the parasites are seen as budding cells and mycelium. The culture shows a pasty colony. Definite mycelium and moniliform clusters appear in corn-meal agar. Chlamydospores, large, thick-walled globular cells, are also seen. This morphology is characteristic of *Monilia albicans*. An identical species is found in thrush and sprue. It may be differentiated from other species of *Monilia*, *Cryptococcus* and *Saccharomyces* both by its morphologic aspects in corn-meal agar and by the agglutination reactions.

#### CLASSIFICATION OF DERMATOPHYTES. CHESTER W. EMMONS.

The classification of dermatophytes proposed by Sabouraud remains the most workable and the most widely accepted of those used. It is based primarily on the clinical aspects of the lesions. Having determined his genera and subgeneric groups in this manner, Sabouraud bases his specific characters largely on the

appearance of the giant colony. A natural classification based on the morphologic characters of the fungi would be desirable. Classifications proposed by Ota and Langeron, Guiart and Grigorakis, and others attempt such natural groupings. The attempts so far made are not satisfactory, because of the variations and intermediate spore forms that appear, and because of the dependence to some extent on quantitative rather than on qualitative differences.

Three series of variations appearing in three strains of dermatophytes are presented. In a strain of *Achorion gypseum* which arose from a single spore, six variants appeared in an old culture. They differ in microscopic and macroscopic characters from the parent strain and from each other sufficiently to deserve specific ranks according to the usual criteria. Shorter series of variations appeared in two strains of *Trichophyton gypseum*.

The dermatophytes appear to comprise a closely related group of fungi. It is hoped that a careful study of the characters held in common by different strains and of variations that appear in strains arising from single spores will aid in reducing the number of some of the imperfectly described "species" and contribute to a more satisfactory classification of these fungi.

#### DISCUSSION

PAUL KLEMPERER: Are the tissue reactions found in animals experimentally infected with pathogenic fungi in any way characteristic of or similar to the lesions provoked by the yeasts in the few cases that are seen in man? This question is asked particularly with reference to the one case that Dr. Hopkins referred to. This was a puzzling case for us, in which the histologic picture of the skin lesion did not in any way conform with the lesion that we learned to recognize as produced by yeast, in which the tubercle-like production is characteristic. I wonder if the reactions in animals are so variegated as those we see in human beings, or are they more uniform?

MAURICE RICHTER: I should like to ask whether morphologic variations in these organisms can be correlated with variations in virulence.

J. GARDNER HOPKINS: We have not been impressed in the few histologic specimens that we have had of sporotrichosis and blastomycosis with any characteristic tissue reaction. From the descriptions I have seen, I think it extremely difficult to make any other diagnosis than that of chronic granuloma.

CHESTER W. EMMONS: The strain of *Achorion gypseum* in which the longest series of variants appeared is not pathogenic for the guinea-pig, so that it was not possible to get a good series of inoculations, but in both strains of *Trichophyton gypseum* that showed the variants these were pathogenic for guinea-pigs, perhaps not quite so severely as the parent strains, but they produced definite lesions.

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## AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

C. PHILLIP MILLER, *Secretary*

*Nineteenth Annual Meeting, The University of Pennsylvania,*

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SAMUEL R. HAYTHORN, *President, Presiding*

REGENERATION OF HEMOGLOBIN AS MODIFIED BY ABNORMAL CONDITIONS OF THE LIVER. F. S. ROBSCHT-ROBBINS and G. H. WHIPPLE, University of Rochester, School of Medicine and Dentistry.

A simple anemia was produced in dogs by withdrawal of blood. This anemia was maintained at a hemoglobin level of from 40 to 50 per cent for a period

of years. The hemoglobin output due to various diets was carefully evaluated and was constant as shown by repeated experiments during the entire period of the anemia.

With these observations as a base-line control, the regeneration of hemoglobin was determined under abnormal conditions of the liver, such as Eck fistula. Interesting differences have been noted in the reactions of the anemic dog with Eck fistula to standard dietary regimens. The liver in the dog with Eck fistula was further injured by chloroform and the regeneration of hemoglobin studied under these conditions.

PRODUCTION OF OSTEITIS FIBROSA WITH OVERDOSES OF VITAMIN D. ROBERT C. GRAUER (by invitation, introduced by Samuel R. Haythorn), Singer Memorial Laboratory, Pittsburgh.

Seven groups of adult guinea-pigs weighing about 500 Gm. were given viosterol in graded doses. The viosterol used was specially prepared so that 1 cc. of the oil contained 5 mg. of the activated sterol. The animals were kept on the regular laboratory ration of hay, leafy greens and white bread. The guinea-pigs were killed and examined at approximately seven day intervals, and sections were taken from the long tubular bones and from the costochondral junctions of the ribs. These were fixed in Zenker's fluid, decalcified and stained with phloxin and methylene blue (methylthionine chloride, U. S. P.).

No changes were noted in the guinea-pigs given doses under 10 mg. In the groups that received from 10 to 15 mg., bony changes were observed after the twenty-fifth day. There was lacunar resorption in the cortical portions of the long bones. These were lined by active osteoclasts. Endosteal fibrous proliferation with fibrous replacement of the decalcified bone was present. In the group that received 15 mg. of oil for thirty-six days there were areas of hemorrhage and resorption of the hemorrhagic areas with formation of cysts.

The costochondral junction of the ribs revealed thinning of the cortices, spontaneous fractures, fibrous ingrowths and the formation of osteoid tissue. The pictures presented were those of osteitis fibrosa cystica. Studies of the serum calcium, phosphorus and protein revealed only a slight increase over the established normal values.

AMMONIUM CHLORIDE OSTEOPOROSIS ON LOW AND HIGH CALCIUM INTAKES AT DIFFERENT AGES. HENRY L. JAFFE, AARON BODANSKY and J. P. CHANDLER (by invitation), the Hospital for Joint Diseases, New York.

Four litters of dogs, 3, 6, 8 and 18 months old, respectively, were used. Litter mates received a diet that in one case was high and in another low in calcium, with and without ammonium chloride. The diet consisted of fresh lean horse meat supplemented with bone meal and calcium lactate in the case of the dogs receiving the diet high in calcium, and with cod liver oil and tomato juice in all cases. The daily calcium supplement amounted to from 0.5 to 2.5 Gm. Once a week all dogs were fed cooked liver and kidney. The ammonium chloride, in 1 per cent solution, was given by stomach tube. At the end of the experiment as much as 1 Gm. per kilogram of body weight was given daily.

The animals were subjected to the experiment for eleven weeks. The bones in the controls on the diet high in calcium were normal in every respect. The controls on the diet low in calcium showed thinning of the bones.

In all age groups, the gradation of changes was found to be strikingly dependent on the intake of calcium. Dogs receiving a diet high in calcium plus ammonium chloride showed less osteoporosis than those receiving a diet low in calcium plus ammonium chloride or a diet low in calcium without ammonium chloride. In the younger age groups, the diet low in calcium plus ammonium chloride brought out more striking changes, including fractures and deformities, than the diet low in calcium without ammonium chloride.

BONE LESIONS IN RATS PRODUCED BY THE SUBSTITUTION OF BERYLLIUM FOR CALCIUM IN THE DIET. SHELDON A. JACOBSON (introduced by H. L. Jaffe), the Hospital for Joint Diseases, New York.

Experiments on the basis of the work of Branion, Guyatt and Kay were undertaken as follows: All animals except those of the first group received three drops of cod liver oil daily.

Young rats on the unsupplemented Steenbock diet became rachitic. A control group on the Steenbock diet plus cod liver oil had normal-appearing bones. On a calcium-free Steenbock diet, however, marked osteoporosis was manifest. When, for the calcium of the diet, beryllium was substituted, a lesion anatomically similar to marked rickets appeared. When, however, only one half of the calcium was replaced by beryllium, rickets at first appeared, but subsequently showed a tendency toward healing. There was some osteoporosis.

A straight meat diet brought about the appearance of severe osteoporosis. The addition of beryllium to this regimen resulted in a rachitoid lesion being superimposed on the osteoporotic condition of the bones. When both beryllium and calcium were administered, an initial rachitoid lesion developed, but subsequently resolved; the osteoporosis, however, remained.

THE REPAIR OF ARTICULAR CARTILAGE AND THE REACTION OF NORMAL JOINTS OF ADULT DOGS TO SURGICALLY CREATED DEFECTS OF ARTICULAR CARTILAGE, JOINT MICE AND PATELLAR DISPLACEMENT. GRANVILLE A. BENNETT, WALTER BAUER and STEPHEN J. MADDOCK, Harvard Medical School, Massachusetts General Hospital and Boston City Hospital.

Reparative changes were demonstrable in all but two of seventeen surgically created defects of cartilage. Such repair was manifested in one of three ways: by independent regeneration of original cartilage, by proliferation of connective tissue from the subchondral marrow spaces, or by the ingrowth of pannus from the marginal synovial membrane. A limited ability of cartilage to repair aseptic lesions made entirely within cartilage, by multiplication of original cartilage cells was observed in several of the defects. More active and more nearly complete regeneration of cartilage occurred on the weight-bearing surface of the femoral condyle than on the nonweight-bearing surface of the patellar groove.

In contrast to conclusions of others it was found that neither the presence of surgical defects in cartilage or cartilage and subchondral bone nor the presence of "joint mice," consisting of cartilage or cartilage with attached bone, was a cause of important associated joint changes.

In each joint in which permanent patellar displacement occurred, there were marked intra-articular changes of a type similar to the changes encountered in human hypertrophic arthritis. Such changes became prominent within four weeks and progressed.

THE ANTERIOR LOBE OF THE PITUITARY GLAND AND HYPERTHYROIDISM. LEO LOEB, Washington University.

The effects of administration of extracts of the anterior lobe of the bovine pituitary gland to guinea-pigs are compared with the changes observed in cases of exophthalmic goiter in man.

A RAPID PHYSIOLOGIC TEST FOR THE CORPUS LUTEUM HORMONE. G. W. HART and J. A. MORRELL (introduced by John F. Anderson), New Brunswick, N. J.

A technic is described whereby it becomes possible to complete the physiologic assay of any corpus luteum preparation within five days. The uterus of the rabbit is sensitized by the induction of ovulation as the result of a single intravenous injection of urinary hebin. From twenty to twenty-four hours later, the

animal is castrated, and twenty hours later a subcutaneous injection of the extract to be tested is given and repeated every twelve hours until four injections have been given. Approximately forty-eight hours after the first injection, the rabbit is killed by a blow on the occiput. The uterus is removed and transferred to a tube containing warm oxygenated Locke's solution. When the regular contractions have been fully established, 0.2 unit of extract of the posterior lobe of the pituitary gland in 1 cc. of physiologic solution of sodium chloride is introduced into the Locke solution, and any change in the rate and nature of the contractions is noted. An active extract containing the corpus luteum hormone causes a uterus under these conditions to show either no response to the extract of the posterior lobe of the pituitary gland or some markedly modified response. There is a certain parallelism between the type of response obtained and the degree of proliferation of the uterine mucosa. We have found that when no response to the extract of the posterior lobe of the pituitary gland is obtained, this dosage is equivalent to two Corner rabbit units.

DUODENAL ULCER FOLLOWING DAMAGE TO THE ADRENAL GLANDS. CHARLES McLAUGHLIN (introduced by I. S. Ravdin), University of Pennsylvania.

In a series of dogs, the adrenal glands were damaged by a high frequency coagulating current. The major injury resulted to the cortex, but the medulla was affected. The operation was done in two stages. In fifteen of nineteen animals duodenal and jejunal ulceration occurred. The ulceration was of two types; the one was limited to superficial erosion, while the second was characterized by the heaping margins of the chronic ulcer of man. With the method used, gastric ulceration did not occur.

SOME PHYSIOLOGIC EFFECTS OF ACETONE. M. M. KUNDE, University of Chicago.

Acetone in quantities of 2 cc. per kilogram causes an increase in the basal metabolic rate and marked hyperglycemia. Removal of one adrenal gland and denervation of the other prevent the hyperglycemia but do not prevent increase in the basal metabolic rate.

EFFECTS OF TOTAL REMOVAL OF THE LIVER IN THE MONKEY (MACACUS RHESUS). STEPHEN MADDOCK and ANDREA SVEDBERG (by invitation), Boston City Hospital.

The animal was prepared for removal of the liver by preliminary ligation of the portal vein and vena cava as described by Markowitz and Soskin. The results of studies on twenty-five animals are included in the report. Biochemical studies were made using both the Folin-Wu and the Folin method of precipitation. The animals lived for periods varying from twelve to eighteen hours. The results are entirely comparable with the findings previously reported for the dog and the rabbit.

SPLENECTOMY IN DOGS WITH BILIARY FISTULA; OVERPRODUCTION OF BILIARY PIGMENT. F. B. QUEEN (by invitation), W. B. HAWKINS (by invitation) and G. H. WHIPPLE, University of Rochester.

The output of biliary pigment in a healthy dog with biliary fistula is somewhat constant, but can be modified by certain diets and fluctuates at times owing to unknown factors. A splenectomized dog with biliary fistula may run the usual course of a normal control with biliary fistula for some time, but after a period of weeks or months the splenectomized animal develops cycles of anemia and an extraordinary excretion of biliary pigment. After one or more cycles, the dog dies of anemia or of intoxication. The output of biliary pigment may increase to from five to seven times the normal level. The drop in hemoglobin is entirely

inadequate to explain this great increase in excretion of biliary pigment as due to destruction of hemoglobin and resultant formation of biliary pigment. The hemoglobin does not return to normal levels nor does the biliary pigment fall to the normal base line between these cycles. So far as we know, this is the only condition in which the spleen is essential to life. It indicates that the spleen and the bile together are essential for normal metabolism of pigment. The source of this great excess of biliary pigment is obscure, but the excessive pigment can scarcely be explained as derived only from broken down hemoglobin. This has an important bearing on the understanding of the production of the pyrrole nucleus within the body and its utilization to form either hemoglobin or biliary pigment.

THE EFFECT OF TEMPERATURE ON THE PERMEABILITY OF RESTING AND OF ACTIVATED CELLS TO WATER. MORTON MCCUTCHEON and BALDUIN LUCKÉ, University of Pennsylvania.

It has been shown that the permeability of the living cell to water, like other properties of the cell, varies with temperature. When an isolated cell, such as the spherical egg of the sea urchin (selected because it can be measured accurately), is exposed to hypotonic solutions, water enters the cell more rapidly at higher than at lower temperatures. In the case of the resting (unfertilized) egg, permeability to water, defined as the rate of passage of water per unit area of surface per unit of pressure, has a temperature coefficient approximating a  $Q_{10}$  value of 2.7. This value is much higher than would be the case if the passage of water into the cell were purely a process of diffusion. It indicates that with change in temperature there is change in the resistance of the surface of the cell to flow of water. Such a change might be either reversible or irreversible (owing to injury), as previous experiments have shown that injury due to heat is manifested by increased permeability to water. To determine whether the temperature effect is reversible, permeability was determined first at a higher temperature, then at a lower; with other cells the procedure was reversed, measurements being made first at the lower, then at the higher temperature. Permeability was found to be the same at corresponding temperatures, indicating that the effect of temperature is entirely reversible. At the end of each experiment, the eggs were inseminated; subsequently normal cleavage occurred, showing that the cells had not been injured. Activated (fertilized) eggs, which are more permeable to water than are unfertilized eggs, yielded an even higher temperature coefficient, a  $Q_{10}$  value of about 3.5.

NORMAL AND PATHOLOGIC PERMEABILITY OF LYMPHATIC CAPILLARIES. STEPHEN S. HUDACK (by invitation) and PHILIP D. MCMASTER, Rockefeller Institute for Medical Research.

Intracutaneous injections in man are predominantly intralymphatic injections, as shown by the use of isotonic solutions of vital dyes. After injection, diffusible vital dyes pass rapidly from the minute lymphatics of the skin to the interstitial tissue. Lymphatic drainage in the normal human being is so rapid as to carry dye from the lower forearm to the axilla in from ten to twelve minutes and render the major lymphatic trunks visible along the route.

When the dye solution is injected at the edge of the ear of the mouse, the lymphatics draining the spot become visible practically at once because of their colored content. The method has made it possible to test the permeability of the wall of the lymphatic capillary. The rapidity with which the dye spreads, secondarily from these vessels to the surrounding tissue varies directly with the diffusibility of the dye, other things being equal. Dyes that fail to pass through the wall of the blood capillary are held back also by the wall of the lymph capillary. Slight causes suffice to produce great alterations in the permeability of the lymphatic vessels. Slight mechanical, thermal or chemical stimulation greatly increases the permeability.

CORRELATION OF MITOCHONDRIAL ALTERATIONS IN RENAL EPITHELIUM WITH SECRETORY ACTIVITY AS DETERMINED BY THE EXTRAVITAL METHOD.  
JEAN OLIVER, Long Island College of Medicine.

Two recent additions to methods of study of the kidney have made possible a new attack on the problem of the part played by the mitochondria of the renal epithelium in functional activity. These are (1) the demonstration that neutral red is secreted by the tubule cells (Scheminsky, F.: *Arch. f. d. ges. Physiol.* **221**:641, 1929. Oliver, J., and Shevsky, E.: *J. Exper. Med.* **50**:15, 1929), and (2) the observation that anatomic processes can be studied extravitally in the perfused organ (Oliver, J., and Smith, P.: *ibid.* **53**:785, 1931).

The kidneys of a single animal, the frog, were compared with respect to the mitochondria. The one kidney was functioning under perfusion in such a manner that the secretory activity, as shown by examination of the urine, was low if not entirely absent, while in the other the epithelial cells were actively secreting the dye, neutral red, in large amounts.

It was found that the anatomic expression of these two different functional states is a replacement of the filamentous mitochondrial structures of the non-secreting epithelium by granulovascular structures in the secreting epithelium. A further study of these granulovacuolar bodies by means of vital, supravital and extravital staining with janus green and neutral red showed that their material, although its fundamental reactions are those of mitochondrial substance, has acquired added characteristics not found in the original mitochondria.

There have been demonstrated, therefore, under rigorously controlled experimental conditions, not only structural alterations in the mitochondria during secretory activity, but also changes of some physical or chemical nature.

Similar relations between secretion and mitochondrial alterations were then demonstrated in the living frog under the physiologic conditions of its natural life.

SULPHYDRYL—ITS EFFECTS ON CELL ORGANIZATION AND DIFFERENTIATION AND THE RELATIONS TO NEOPLASIA. STANLEY P. REIMANN, Lankenau Hospital Research Institute, Philadelphia.

Sulphydryl stimulates cells to divide beyond the normal rate for the species. This higher rate has been correlated directly by Hammett (*Protoplasma* **13**:331, 1931) with the higher differentiation and organization that have been found. Since neoplasias exhibit degrees of differentiation and organization lower than normal for the species, phenomena of cell division alone cannot be responsible for neoplasias. The division must occur in a cell that is constitutionally altered in its organization and differentiation factors. Whether this alteration is in the chromosomes or in the "working" nucleus or in the cell as a whole remains to be discovered. At all events, these experiments show that normal cells stimulated by the naturally occurring stimulus differentiate and organize more; therefore, for less to occur requires that the cells undergoing division be abnormal.

THE ORIGIN OF THE INFILTRATING CELLS IN TRANSMISSIBLE LYMPHATIC LEUKEMIA OF MICE. JAMES S. POTTER (by invitation) and MAURICE N. RICHTER, Carnegie Institution of Washington, Cold Spring Harbor and Columbia University.

Fourteen transmission lines of lymphatic leukemia of mice have arisen in this laboratory by transfer from as many spontaneous cases. Investigations into the nature of the agent have led to a study of the origin of the infiltrating cells in several of the transmission lines.

• Since genetic uniformity of the hosts assures 100 per cent susceptibility, and since there are characteristic distribution of lesions and a characteristic interval before death after injection of a given line, it is possible to trace the injected cells



and their descendants in serial sections of tissues taken from animals killed at intervals following inoculation.

The predominating type of cell in the infiltrations of a given line is constant through numerous transfers. The infiltrating cells can be traced to an origin in cells injected into the host. Lesions form and enlarge by infiltration of cells that subsequently divide by mitosis.

During the formation of lesions there is no observable increase in the formation of lymphocytes by the tissues of the host.

The conclusion is reached that the infiltrating cells of a given line of inoculable lymphatic leukemia of mice are descendants of the cells of the original spontaneous case.

THE EFFECT OF MASSIVE EXPERIMENTAL HEMORRHAGES ON THE DOG'S "ERYTHRONE." E. B. KRUMBHAAR and A. R. CAMERO (by invitation), University of Pennsylvania.

The effect of massive hemorrhages was studied with special reference to the size of the red blood cell. Two of the four test animals were bled of 50 per cent of their blood volumes. From a third dog 60 per cent was removed on one day and an additional 30 per cent of the remaining blood on the next. The fourth dog was bled of 50 per cent on one day and of 30 per cent of the remainder on the next. Two intact dogs were used as controls. Direct observations included counts of erythrocytes and reticulocytes, estimations of hemoglobin (Sahli), hematocrit readings, determinations of plasma volume and direct measurement of the diameters of fresh red blood cells with the ocular micrometer. The total number of erythrocytes measured in this way was 13,200. From these direct observations, total cell volume, total blood volume and mean corpuscular volume (Wintrobe) were calculated.

Following the loss of 50 per cent of the blood volume, there was a striking increase in the "scatter" of erythrocyte diameters, with many large cells and almost as many small ones, resulting in only a slight increase in the average diameter. The dogs subjected to maximum hemorrhages (approximately 80 and 90 per cent of the blood volume, respectively) showed a definite increase in average diameter of cells as well as an increase in "scatter." At the time of maximum posthemorrhagic change, the average diameter of the cells was found to be 8.3 microns, with cells ranging from 6.3 to 11.3 microns. The normal average diameter by the method used had been found to be 7.7 microns, with cells ranging from 6.1 to 9.4 microns. In all of the test animals, the calculated mean corpuscular volume reflected somewhat more strikingly the increase in the size of the erythrocyte. The maximum changes were noted between the second and the fourteenth day after the hemorrhages.

There were an expected fall in the erythrocyte count and hemoglobin percentage, a noteworthy reticulocyte rise, a fall in total cell volume, an increase in plasma volume and a more or less constant total blood volume. The blood picture returned to normal after eight weeks.

BONE MARROW IN NEUTROPENIC STATES AND THE PROBLEM OF MYELOID STIMULATION. CHARLES A. DOAN, Ohio State University.

Experimental studies, begun in 1924 and published in 1928, established the direct action of the nucleinate molecule, or of the nucleotides comprising it, on bone marrow in effecting a striking peripheral leukocytosis. More recent studies have shown that daily injections of the nucleotides into the rabbit for a brief period (one week) produces hyperplasia of the myeloid tissues in the marrow, with a gradual increase in the number of granulocytes in the peripheral blood.

The long continued (four months) daily injection of large quantities (1 Gm.) of sodium nucleinate produced a high level of mature granulocytes in the peripheral circulation throughout the experiment, and post mortem, in addition to generalized

hyperplasia of myeloid cells in all bone marrow, ectopic foci of developing myelocytes were found in both kidneys and spleen. The rationale for this action of nucleic acid is in the occurrence of "nonmotile" showers of granular leukocytes under physiologic and most pathologic conditions, with the possibility of the nuclear products thus liberated acting as the normal replacement stimulus in the mechanism of cellular hemostasis.

The results of examination of the blood in two clinical cases of malignant neutropenia in which nucleotide therapy was used will be presented to illustrate the response of the human myeloid mechanism; in the first instance, from a marrow in which foci of myelocytes were still present, even though there were no neutrophilic leukocytes in circulation; in the second, from a marrow hypoplastic or essentially aplastic for myelocytes, but in which their regeneration was stimulated and the subsequent myelocytic maturation followed through careful cytologic studies of the cells appearing in the blood during recovery.

A hypothesis suggesting a factor common to all three procedures—irradiation, blood transfusion and the injection of nucleotide—now advocated in meeting the therapeutic problem of the Schultz syndrome may explain the mechanism by which some clinical recoveries have followed each method of treatment, and may suggest when each is indicated.

SULPHIDE ANEMIA: NONSPECIFIC ACTION OF ANTI-ANEMIC SUBSTANCES.  
O. M. GRUZHIT, Research Laboratories, Parke, Davis & Company.

The feeding of synthetic monosulphide and disulphide compounds of the general formula  $R-SH$  or  $R-S-S-R$  to dogs produces in them a severe hemolytic anemia. The anemia is similar to that induced by the feeding of onions and the phenylhydrazine type of compounds. The degree of anemia induced by the sulphides is directly related to the quantity administered. Prolonged feeding may lead to the development of a slight tolerance for the drug, which is readily overcome by an increase in the dosage. Withdrawal of sulphide compounds is followed by recovery to an apparently normal hematic condition. In some animals maintained for over a year on normal propyl disulphide there apparently developed a certain degree of aplasia of the red cell degenerating centers, as the normal level of the blood was not attained inside of several months after withdrawal of the disulphide. A probable aplasia was also noted in one case of human polycythemia vera in which the drug was administered for 109 days until the red cell count was reduced from 10,000,000 to 4,700,000 red cells and hemoglobin from 137 to 100 (Sahli). The patient has not had the drug now for 68 days, and the red cell count and hemoglobin have remained stationary at 4,700,000 and 90 (Sahli).

The ease with which anemia could be induced and maintained by administration of sulphide suggested a use of this type of anemia in the study of anti-anemic substances. On the whole, the early evidence tended to show that substances active against Addison's anemia, such as ventriculin and liver extract, were also active against sulphide anemia. Further studies brought out that this action was nonspecific. Feeding of nonspecific substances, such as iron, ash of liver, lean meat, autoclaved ventriculin or liver extract, at times overcome the sulphide anemia and at other times did not. Injections of crude insulin, with or without liver extract, ameliorated the sulphide anemia. Intravenous and intramuscular administration of potent liver extracts as well as harmless brilliant vital red dye was followed by depression of the hematopoietic system.

CERTAIN QUANTITATIVE ASPECTS OF THE CLOTTING OF THROMBIN. E. D. WARNER (by invitation) and H. P. SMITH, University of Iowa.

The clotting time of thrombin has been used especially as a measure of the concentration of thrombin. To measure thrombin in this way one must be certain that other variables have been eliminated. We have been studying a number of the variables and have been trying, as far as possible, to gain a quanti-

tative concept of the importance of each. Such studies should form a sound basis for further work on clotting.

The present work shows that the clotting time varies markedly with the amount of fibrinogen, as well as with the amount of thrombin.

When we plot clotting time, as ordinate, against the concentration of fibrinogen as abscissa, we obtain a U-shaped curve, with the minimum clotting time occurring when the fibrinogen concentration is optimal. With smaller or with larger amounts of fibrinogen, clotting is delayed. The optimal fibrinogen concentration varies somewhat with the amount of thrombin present. To use clotting time as a measure of thrombin concentration, the amount of fibrinogen in the clotting mixture should be controlled.

TWO-COLOR LANTERN SLIDES BY PHOTOMICROGRAPHY. JOHN C. BUGHER (by invitation) and C. V. WELLER, University of Michigan.

A method has been developed in our laboratory for the production of photomicrographs and particularly lantern slides in red and blue without the use of color plates, which have never been fully satisfactory, because of their lack of transparency. By this method, which has been devised by Dr. Bugher, highly transparent lantern slides can be produced in two colors that reproduce satisfactorily the color values of ordinary hemalum and eosin stains.

THE EFFECT OF FASTING OF THE HOST ON CESTODES. DAVID L. BELDING, Boston University.

During its spawning migration in American waters, the adult Atlantic salmon ceases feeding from four to six months. During this period of fasting, certain physiologic changes take place in the salmon, chiefly in the reproductive organs, muscles and digestive tract. At the beginning of the fast, the incidence of tapeworm infection was 40 per cent. After marked atrophy had occurred in the digestive tract, the incidence fell to 9 per cent. During the period of fasting, the salmon had succeeded in ridding themselves of the cestodes, apparently a unique record in the annals of parasitology. (Slides were shown.)

THE PORPHYRIN PIGMENTS OF BACTERIA. CALVIN B. COULTER, Columbia University.

Various procedures of extraction have been carried out in the effort to obtain cytochrome or its derivatives from bacteria. Brewer's yeast, *Bacillus phosphorescens* and *Coryne bacterium diphtheriae* have been used. Extraction of yeast and *B. phosphorescens* with alkali yields a hemochromogen similar to the "cytochrome C" of Keilin. Alkaline extracts of *C. diphtheriae* differ slightly from this with respect to the position of the main absorption bands. From these extracts as well as from the whole bacteria there have been obtained, by extraction with acetic acid-ether, alpha-hematin and a hemochromogen apparently identical with a porphyrin compound that is found in toxic filtrates of cultures of *C. diphtheriae*.

This compound has a characteristic absorption spectrum of two bands in the green. It is very labile and breaks down to yield coproporphyrin and the copper compound of coproporphyrin, and may represent a form in which copper is contained within the cell. The amount of this compound has been found to be proportional to the amount of toxin.

QUESTION: Is it then possible to determine the amount of toxin with the spectroscopy?

ANSWER: One can estimate the amount of toxin in a culture, even without filtration, by proper spectroscopic examination. With the spectrophotometer we have been able to titrate the toxin. The results are reliable, but do not give as high precision as the titration by inoculation of animals.

SPECIFICITY OF STREPTOCOCCI ISOLATED IN STUDIES OF INFLUENZA. EDWARD C. ROSENOW, Mayo Foundation.

The results of experiments in a restudy of the etiology of influenza will be reported. Evidence of specificity of the streptococcus and other bacteria isolated, aside from the usual cultural reactions, was obtained by inoculating animals in various ways with living cultures and the corresponding filtrates and dead bacteria, and with suspensions and filtrates of nasopharyngeal washings and sputum. The cataphoretic velocities of the streptococcus and other bacteria were also determined, which served as a reliable check in establishing whether cultures were influenzal or not.

The more important symptoms and lesions of influenza, such as prostration, leukopenia, pharyngitis, bronchitis, emphysema and hemorrhagic edema of the lungs, massive hemorrhagic, coalescing bronchopneumonia and empyema, have been reproduced in suitable animals.

THE OCCURRENCE OF BACTEROIDES IN THE FECES. ARNOLD H. EGGERTH and BERNARD GAGNON (by invitation), Long Island College of Medicine.

From 50 to 99 per cent of the organisms grown from normal feces were nonspore-bearing anaerobic bacilli of the genus *Bacteroides*. The following species, all but two of which are new, have been isolated and studied:

I. GRAM-NEGATIVE BACTEROIDES

- A. No gas from peptone
  - B. Acid in arabinose and salicin
    - C. Acid in mannitol.....*B. gulosus*
    - CC. No acid in mannitol
      - D. Acid in rhamnose
        - 1. Not encapsulated.....*B. thetaiotaomicron* (Distaso)
        - 2. Encapsulated .....*B. variabilis* (Distaso)
      - DD. No acid in rhamnose.....*B. uniformis*
  - BB. Acid in arabinose; no acid in salicin
    - C. Gelatin liquefied.....*B. vulgatus*
    - CC. Gelatine not liquefied.....*B. incommunis*
  - BBB. Acid in salicin; no acid in arabinose
    - C. Acid in xylose.....*B. distasonis*
    - CC. No acid in xylose.....*B. uncatus*
  - BBBB. No acid in arabinose or salicin
    - C. Acid in sorbitol.....*B. tumidus*
    - CC. No acid in sorbitol
      - D. Acid in rhamnose
        - 1. Acid in lactose.....*B. ovatus*
        - 2. No acid in lactose.....*B. vescus*
      - DD. No acid in rhamnose
        - 1. Acid in xylose.....*B. convexus*
        - 2. No acid in xylose.....*B. exiguus*
- AA. Gas from peptone
  - B. Acid in lactose
    - 1. Acid in arabinose.....*B. inaequalis*
    - 2. No acid in arabinose.....*B. insolitus*
  - BB. No acid in lactose; acid in dextrose....*B. varius*
  - BBB. No acid in dextrose
    - 1. Milk coagulated.....*B. coagulans*
    - 2. Milk not coagulated.....*B. siccus*

II. GRAM-POSITIVE BACTEROIDES

(Work on this group is incomplete and will be reported at a later time.)

TYPES OF RESPONSE IN LABORATORY ANIMALS TO HUMAN STRAINS OF *ASPERGILLUS*. GEORGE H. ROBINSON and SAMUEL R. HAYTHORN, Pittsburgh.

Cultures of *Aspergillus fumigatus* recovered from two infected patients were injected into laboratory animals and the lesions studied. Subcutaneous injection produced abscesses, which later formed pseudotubercles with typical Langhans' giant cells. Intraperitoneal inoculation led to the formation of pseudotubercles, which in some instances broke down and contained central masses that closely resembled actinomycosis. Intratracheal inoculations produced chronic bronchiolitis, pseudotuberculosis and diffuse interstitial pneumonia with lymphoid hyperplasia and formation of germ centers.

Pseudotuberculosis due to aspergilli is well known and is so suggestive of tuberculosis as to make differential diagnosis difficult. The lesions resembling actinomycosis are less well known, but are so similar that special staining is necessary for differentiation.

SOME GENERAL CONSIDERATIONS AND A NEW CLASSIFICATION OF VIRUS DISEASES. EARL B. MCKINLEY, George Washington University.

The virus diseases affect man, animals, insects, fowls, plants and fishes, and are characterized by attributes that set them apart from other diseases such as those that are caused by bacteria and animal parasites. Pathologic, epidemiologic and immunologic differences lend strong support to the belief that filtrable or ultramicroscopic viruses are distinct from bacterial forms, either filtrable or non-filtrable. It is unfortunate that the confusion in regard to filtrable forms of bacteria and true filtrable viruses has been so widely disseminated. While the precise nature of viruses and their relation, if any, to other disease-producing agents are not known, the ultimate solution of the many problems will be best served by the most cautious and conservative interpretation of experimental data. Any classification of the viruses and the diseases that they cause should be based on a careful adherence to what is actually established concerning them. When the subject is systematized, one's comprehension of it becomes more orderly, and when one can separate the material and examine parts of it in an objective manner, one's opinion becomes better crystallized. A new classification of virus diseases is presented, based on the presence or absence of inclusion bodies, on transmissibility and on filtrability of the causative agent. It is expected that as new knowledge accumulates certain changes will of necessity be made, but, in its present form, the classification represents progress and improvement over former attempts to classify a group of agents and diseases in a field involving much debatable terrain.

A RECENTLY DESCRIBED VIRUS DISEASE OF PARROTS AND PARRAKEETS DIFFERING FROM PSITTACOSIS. T. M. RIVERS and F. F. SCHWENTKER (by invitation), Rockefeller Institute for Medical Research.

The widespread outbreak of psittacosis in 1929 and 1930 incited experimental work which quickly resulted in the discovery that the disease is caused by an agent capable of traversing bacteria-tight filters. Thus for the first time a filtrable virus indigenous to parrots was found. Since Amazon parrots were believed to be the chief source of the infection, workers in Brazil sought for evidence of psittacosis in their country. Pacheco, Bier and Meyer encountered a disease in parrots that subsequently was shown to be induced by a filtrable agent. According to their reports, the etiologic agent passes Berkefeld N candles, is not cultivable on ordinary mediums, produces areas of necrosis in the liver and spleen in which are found acidophilic nuclear inclusions similar to those seen in herpes febrilis and virus III infections, and is not pathogenic for pigeons, chickens, mice, guinea-pigs or monkeys. Moreover, these workers stated that the clinical picture observed in infected birds is similar to that of avian psittacosis, and they believe

that the strict adaptation of the virus to the Psittacidae accounts for the absence of human psittacosis in Brazil.

Dr. Pacheco sent us some of his virus in order that its activities might be compared with those of a virus known to have caused psittacosis in human beings. This comparison has been made, and we can confirm the results of the experimental work reported by the Brazilian investigators. We do not agree, however, with the conclusion that the strict adaptation of the virus to parrots and parakeets accounts for the absence of human psittacosis in Brazil. On the contrary, our experiments with the virus indicate that another filtrable virus producing disease of parrots has been discovered. Its difference from psittacosis is manifested by the fact that the causal agent is nonpathogenic for mammals and produces lesions in avian livers and spleens in which numerous acidophilic nuclear inclusions are found, instead of the "minute bodies" first described by Levinthal as characteristic of psittacosis.

VACCINATION AGAINST YELLOW FEVER WITH VIRUS FIXED FOR MICE AND IMMUNE SERUM, W. A. SAWYER, S. F. KITCHEN (by invitation) and WRAY LLOYD (by invitation), Yellow Fever Laboratory, Rockefeller Foundation.

After preliminary experiments in monkeys, fifteen persons were actively immunized by a single injection of a dried mixture of the living virus of yellow fever, fixed for mice, and human immune serum, with separate injections of enough additional serum to make up the amount required for protection. One person was similarly immunized by injecting immune serum and dried virus separately. By titration of the serums of several of the vaccinated persons in mice, it was shown that the immunity rose in a few weeks to a height comparable to that reached after an attack of yellow fever, and remained there throughout a period of observation lasting six months. Yellow fever virus could not be recovered from the blood of vaccinated persons or monkeys, except when they had received less than the specified amount of immune serum. Neutralization of the virus by immune serum took place very slowly in vitro at room temperature, and could not have been an appreciable factor in the vaccination. It appeared that the immunizing reaction after the vaccination was a part of an infectious process which also caused leukopenia.

THE CELLULAR REACTION IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA. ESMOND R. LONG and SION W. HOLLEY (by invitation), University of Chicago.

Results previously obtained (Long, Vorwald and Holley: *Am. J. Path.* 7:555, 1931) indicated that the initial response of the center of the cornea to infection with tubercle bacilli of human type was almost confined to polymorphonuclear leukocytes, which phagocytosed the bacilli and concentrated them in localized lesions. The subsequent development of these lesions varied in different animals, being rapid in the guinea-pig, less rapid in the rabbit and slow in the cat. The leukocytes in the lesions were gradually engulfed and replaced by large mononuclear cells. At the same time, vascularization of the lesion was evident. The fact that large mononuclear phagocytic cells became prominent at the site of infection only after vascularization of the cornea seemed to rule out a local origin for these cells, and indicated that they reached the lesion either through the blood stream or in the walls of the new vessels. It might therefore be expected that an infection of the corneal margin in close proximity to existing blood vessels would attract large mononuclear cells much earlier than an infection more remote. This proved to be the case in an experiment in which, in the same rabbit, two small doses of tubercle bacilli were placed, one in the cornea close to the limbus, and one in the center of the organ. After one day the reaction was almost exclusively polymorphonuclear in both locations; at one and two weeks, when vascularization had not yet reached the center, it was mononuclear at the margin

and polymorphonuclear at the center; at six weeks, when the center was vascularized, the reaction was mononuclear in both places. A double origin for the mononuclear cells taking part in the reaction was indicated by the fact that monocytes and lymphocytes were present in increased number in the vascular lumina, and similar cells were at the same time observed as a thick mantle around the newly developing vessels. The majority of the cells in this mantle appeared to come from the lumen of the vessel, as indicated by migratory figures. But mitotic figures in the adventitia indicated an origin from this source also. Various transitional stages were seen in the cells beyond this mantle, including the final stage, the epithelioid cell.

LOCAL SENSITIZATION OF THE SKIN (ARTHUS' PHENOMENON) PRODUCED IN NORMAL RABBITS AND GUINEA-PIGS BY THE PROTEIN OF TUBERCULIN.  
FLORENCE B. SEIBERT, University of Chicago.

Contrary to many previous reports, it has been possible to sensitize the normal guinea-pig and the normal rabbit to the protein of tuberculin so that the animal reacts to an intracutaneous injection of the protein exactly as a tuberculous animal or one that has received an injection of dead bacilli reacts. An inflammatory reaction with edema, induration, leukocytic infiltration and necrosis results in both cases.

This sensitization was brought out with less difficulty than that to crystalline egg albumin. It followed treatment with highly purified tuberculin protein, practically free from reducing substances as well as treatment with the less pure fractions.

When a guinea-pig that had been rendered cutaneously sensitive to the tuberculin protein was given a testicular injection of the homologous antigen, the reaction was similar to the testicular reaction of the tuberculous animal to tuberculin, except that it was possibly less intense.

The relative merits of different methods of preparing and purifying the protein in such a way as to preserve its antigenic capacity were studied. When purified merely by ultrafiltration or precipitated by means of ammonium sulphate or trichloro-acetic acid, the protein has excellent antigenic properties for producing cutaneous hypersensitiveness. When heated, as in the preparation of old tuberculin, the protein loses much of its antigenic properties.

THE DIRECT OBSERVATION IN VITRO OF PHAGOCYTOSIS BY MACROPHAGES AND POLYMORPHONUCLEAR LEUKOCYTES. EMILY B. H. MUDD (by invitation) and STUART MUDD, University of Pennsylvania.

Studies conducted for a number of years by Lucké, McCutcheon, Strumia and ourselves have shown that immune serums promote phagocytosis in proportion as they produce certain definite changes in the cohesiveness, wetting properties and iso-electric points of the particles to be phagocytosed. These changes are such as to indicate that the antibodies are modified serum globulins that combine specifically with and form deposits on the surfaces of the antigen, and that the effect of antibodies in promoting phagocytosis is essentially due to their thus forming surfaces on which leukocytes can spread.

The latter conclusion has been corroborated in the present experiments by direct observation and photography of phagocytosis in vitro by both macrophages and polymorphonuclear leukocytes. Phagocytosis by mammalian white blood cells is essentially a process of spreading; the leukocyte surface spreads on the surface of the particle ingested under the action of interfacial tension.

This process of spreading under surface forces is, however, modified by the resistance to deformation of the protoplasm of the phagocytosing cell. The macrophages are, at least under the conditions of our experiments, more resistant to deformation than the polymorphonuclear leukocytes, and the picture of phagocytosis by the two types of cells differs correspondingly.

FURTHER STUDIES ON THE MECHANISM OF FIXATION BY THE INFLAMMATORY REACTION. VALY MENKIN (introduced by S. Burt Wolbach), Harvard Medical School.

I have recently shown that various foreign substances or bacteria injected into an area of inflammation are fixed *in situ* and fail to drain readily into the tributary lymphatic vessels, while their intravenous injection results in rapid accumulation in the inflamed area. This accumulation is partly the result of the increased permeability of the capillaries, but is also the result of the inability of these substances to escape from the site of inflammation. The retention or fixation of these substances at the site of inflammation has been shown to be due to mechanical obstruction in the form of thrombosed lymphatic vessels and a fibrinous network in tissues distended with edema. In addition to these histologic findings, a second line of evidence to support the hypothesis that mechanical obstruction is the explanation of fixation has been presented by the failure of either dye or bacteria to penetrate the inflamed area when injected at its periphery.

Present studies are concerned with a third group of experiments corroborating further this hypothesis. On the basis of the *in vitro* observation of Foulger and Mills that urea in high concentrations delays or inhibits clotting of blood by "peptizing" fibrin, I have studied the effects of concentrated urea solutions on the fixation of foreign materials at the site of inflammation with the following results:

1. When injected simultaneously with the inflammatory irritant (aleuronat), urea (50 per cent or, in some cases, 30 per cent) inhibits either wholly or in part the fixation of graphite and of iron in the inflamed peritoneal cavity, allowing free or partial dissemination of these foreign substances to the retrosternal lymph nodes. When the injection of urea follows the onset of inflammation produced by aleuronat in the peritoneal cavity, inhibition of fixation of graphite also occurs. The intensity of this inhibition seems to vary in inverse relation to the length of time elapsing between the injection of aleuronat and that of urea.

2. These results have been correlated with histologic findings, inhibition of fixation being associated with the absence of thrombosis in lymphatic vessels. Likewise in some of the experiments in which the addition of concentrated urea to the inflammatory irritant produced only partial fixation of graphite particles, histologic studies revealed small thrombi occluding in part the lumina of the retrosternal lymphatic vessels.

3. When injected into a normal peritoneal cavity, concentrated urea by itself sets up an inflammatory reaction. However, fixation of graphite does not occur. That concentrated urea (20 to 50 per cent) alone causes increased permeability of capillaries is shown by the accumulation of trypan blue from the blood stream in cutaneous areas almost immediately after these areas are subjected to injection of urea. Histologic sections of skin some hours after injection of urea reveal the usual leukocytic reaction accompanied by considerable extravasation of red cells.

4. Penetration of trypan blue when injected into an area of skin at its periphery takes place when the area has previously been subjected either to injection of urea followed by a suspension of cultures of *Staphylococcus aureus*, or to injection of urea alone.

5. Studies *in vitro* show that when inflammatory exudate is added to 50 per cent urea solution, clotting of the exudate is prevented. Addition of concentrated urea solution to coagulated exudate dissolves the coagulum either completely or partially. This solvent action is facilitated if the urea is added very soon after clotting occurs.

These experiments demonstrating the inhibitory effect of concentrated urea on fixation of foreign substances at the site of inflammation, correlated with the histologic findings and with the fact that concentrated urea has been shown to possess a solvent action on fibrin, furnish the third line of evidence as to the rôle of thrombosis of lymphatic vessels and of deposits of fibrin in explaining fixation at the site of inflammation.



LOCAL FORMATION OF ANTIBODIES BY THE NASAL MUCOSA. T. E. WALSH (by invitation), F. L. SULLIVAN (by invitation) and PAUL R. CANNON, University of Chicago.

Previous work by us has shown that injection of antigen into an area of skin in which macrophages have been mobilized may lead to local formation of antibodies. Similar experiments have been performed on the nasal mucous membranes of rabbits, with similar results. Daily instillations or insufflations of a formaldehydized vaccine of *Bacterium paratyphosum* B at daily intervals for at least ten days have led to local formation of specific agglutinins. These have been demonstrated by the simultaneous titration of glycerol-salt solution extracts of these tissues, liver, spleen, blood serum and kidney against a suspension of living cultures of *B. paratyphosum* B. Instillations for less than this period have given inconclusive or negative results. Extracts of the nasal mucosa of normal rabbits contained no agglutinins for *B. paratyphosum* B at a dilution of 1:120 or above. Extracts of nasal mucosa of rabbits immunized subcutaneously showed no agglutinins at a dilution of 1:120, although the titers of spleen, liver and blood serum ranged from 120 to 1,920. The irritation of the nasal mucosa of subcutaneously immunized rabbits by instillations of 1 part of solution of formaldehyde, U. S. P., to 300 parts of physiologic solution of sodium chloride ("normal saline") did not lead to a mobilization or concentration of agglutinins in the nasal mucosa.

We conclude, therefore, that specific agglutinins may be formed locally by adequately repeated application locally of the appropriate antigen.

THE PROTECTIVE ACTION OF ANTIBODY IN IMMUNIZED ANIMALS DEPRIVED OF LEUKOCYTES. ARNOLD R. RICH and CLARA M. MCKEE (by invitation), Johns Hopkins University.

Immunized animals were treated with benzene to remove their leukocytes, and were then inoculated intracutaneously with virulent pneumococci. In the immune, leukocyte-free body, the presence of antibody influences profoundly the character of the growth of the bacteria and the course of the infection. The bacteria as they proliferate adhere to themselves and to the tissues and are thus held fixed at the site of inoculation for hours after controls have died with septicemia. This fixation occurs and can be maintained for forty-eight hours or more in the complete absence of local inflammatory exudate of any sort. It is, therefore, the antibody content of the body fluids, not inflammation, that is responsible for the prevention of immediate spread of pneumococci in the immune body. However, in the absence of leukocytes the local growth of the immobilized bacteria proceeds uninterruptedly, and the animals eventually succumb even when their plasma is potent in passively protecting nonimmune, normal animals. The humoral antibody, therefore, performs the important protective function of preventing the immediate spread of the bacteria throughout the body, but acquired immunity creates no condition of the fluids or of the fixed tissues that can prevent the progressive growth of the bacteria in the absence of the leukocytes.

INFLUENCE OF CONCENTRATION OF SOME ORGANIC SOLVENTS ON PRECIPITATION AND DENATURIZATION OF SERUM PROTEINS AND ANTIBODIES. M. H. MERRILL (by invitation) and M. S. FLEISHER, St. Louis University.

Serum proteins can be completely precipitated at room temperature (22 C.) by concentrations of alcohol or of acetone above 90 per cent. Such precipitates are completely soluble in isotonic salt solution or in distilled water. Various factors, such as hydrogen ion concentration, time and presence of electrolytes, must be considered.

Immune serums thus precipitated by alcohol or by acetone can be dried by further extraction with ether without loss in solubility or in activity.

BASIC PRINCIPLES OF PATHOLOGIC REACTION IN THE BRAIN. WILDER PENFIELD (introduced by I. S. Ravdin), McGill University, Montreal.

The work of many investigators has served to simplify certain aspects of neuropathology. Neuroglia and microglia are delicate indicators of the functional condition of the brain. The chief pathologic forms of these cells are outlined in the hope that pathologists may utilize more widely the basic principles underlying these changes as a guide to an understanding of the condition of the brain even when the primary pathologic process may be situated elsewhere.

These principles may be used as a guide without allowing recent confusing contributions to cloud the picture until time has been allowed for substantiation or disproof.

THE ISOLATION OF THE ORGANISM OF *BARTONELLA MURIS* ANEMIA. DAVID PERLA and J. MARMORSTON-GOTTESMAN (introduced by David Marine), Montefiore Hospital.

An organism has been isolated from the blood of splenectomized anemic albino rats which we believe to be the etiologic agent of *Bartonella muris* anemia. The organism is obtained in pure culture on Noguchi's leptospira medium at room temperature after an incubation period of twelve days. It is gram-negative, actively motile on solid and semisolid mediums and measures from 0.4 to 3.2 microns in length and from 0.2 to 0.4 micron in width. In the initial culture it grows very slowly. After repeated subcultures and passages through animals it grows on the usual enriched culture mediums without blood within forty-eight hours. Neither acid nor gas is formed on twelve of the ordinary sugars. On solid mediums, it forms glistening pinpoint colonies that soon coalesce and form a tenacious growth over the surface. Blood cells in culture mediums are not hemolyzed. Cultures on solid mediums have a characteristic sweet pineapple odor.

The organism is nonpathogenic for adult rats of carrier stock, adult rabbits and adult guinea-pigs. A severe anemia was produced in fourteen 3 week old rats, three 3 week old guinea-pigs and three 3 week old rabbits by intraperitoneal injections of 0.5 cc. of the culture from leptospira medium. The organism was reisolated during the course of the anemia from the blood, liver and spleen. Occasionally bartonella bodies were found in the red cells. The blood of these animals was infectious for animals of the same age. After repeated passages through animals, the organism showed enhanced virulence and a moderate anemia was produced in Wistar splenectomized adult rats of noncarrier stock. There is a striking similarity of *Bartonella muris* to *Bartonella bacilliformis* of Oroya fever culturally as well as morphologically, and the identity of the two organisms is suggested.

A TUMOR-LIKE CONDITION IN RABBITS INDUCED BY A FILTRABLE AGENT. RICHARD E. SHOPE (introduced by Carl Tenbroeck), Rockefeller Institute for Medical Research, Princeton, N. J.

From subcutaneous tumors of a wild cottontail rabbit has been secured an agent that, when injected subcutaneously or intratesticularly into the domestic or the wild rabbit, produces at the site of inoculation a large, firm, tumor-like mass composed of rapidly growing connective tissue cells. This mass reaches its maximum size in from ten to twenty days in the domestic rabbit and then slowly regresses. It persists much longer in the wild rabbit. No metastases have been detected. The disease is never fatal, and there are no systemic symptoms. It is transmissible in series. One infection renders a rabbit immune, and serum from such an immune animal neutralizes the etiologic agent. The agent passes Berkefeld V and N filters and resists glycerination at the temperature of the refrigerator for at least eighty-six days. It is not demonstrable in the blood stream. Intravenous and intraperitoneal injection of the agent into rabbits is without effect. White rats and mice, guinea-pigs and chickens are not susceptible to infection

with it. Sections of the skin overlying the tumors in the original animal showed lesions of the epidermis strikingly like those of *Molluscum contagiosum*. Similar lesions have not been observed in inoculated animals. This tumor-like disease bears a peculiar relationship to infectious myxoma. Although the clinical and pathologic pictures are very different, and although serum from a rabbit in which the tumor has regressed fails to neutralize the virus of infectious myxoma, a rabbit convalescent from the tumor-like disease is resistant to infection with the virus of infectious myxoma.

THE CIRCULATION OF THE LIVER IN RELATION TO RESTORATION FOLLOWING PARTIAL REMOVAL. FRANK C. MANN, Mayo Foundation.

One of the most outstanding characteristics of the liver is its ability to undergo restoration after injury or partial removal. A physiologic need for hepatic tissue has usually been the factor to which is ascribed the quick and complete restoration of the liver after partial removal. The results of various investigations by my associates and myself indicate that the major factor causing restoration of the liver after partial removal is the portal circulation. This statement is based on the following facts: (1) restoration in the rat is decreased following partial occlusion of the portal vein; (2) restoration usually does not occur in a dog with Eck fistula; (3) restoration usually does not occur in the chicken, in which species there is a natural anastomosis between the portal and the systemic venous system; however, if the flow of blood through the liver of the chicken is increased by occlusion of the postcava, restoration occurs to a marked degree; (4) restoration does not occur in a dog in which a stoma between the vena cava and the portal vein is made without ligation of either vessel.

These results indicate that restoration of the liver after partial removal is owing to the need of restoring the portal pathways. The relationship of these observations to pathologic conditions of the liver is considered.

THE MEGALOBLAST AS A NORMAL STAGE IN THE DEVELOPMENT OF THE ERYTHROCYTE. RAPHAEL ISAACS, Simpson Memorial Institute for Medical Research, Ann Arbor, Mich.

Many hematologists, including Naegeli and Piney, following Ehrlich, consider the megaloblast as entirely separate from the precursors of the normal red blood cell, absent from normal adult bone marrow, present in the fetus, and reappearing in pernicious anemia. Recent studies of films made from serum suspensions of bone marrow showed that the megaloblast (with reticular chromatin pattern) was present in the sternal bone marrow of more than fifty consecutive patients suffering from many different diseases. In a specimen of normal marrow, the number was 38,700 per cubic millimeter. In pernicious anemia there is a marked diminution in number as long as adequate liver or ventriculin therapy is given, but the number increases during the relapse. With the use of this therapy, there is first an increase in the number of megaloblasts in the peripheral circulation followed by an increase in the number of normoblasts, then in that of the reticulocytes and finally in that of the adult erythrocytes. In films made from serum suspensions of bone marrow, all types of transitional stages between the megaloblastic and the normoblastic cell can be demonstrated.

EXPERIMENTAL MASSIVE ATELECTASIS BY BRONCHIAL STENOSIS AND ITS EFFECT ON PULMONARY TUBERCULOSIS IN DOGS. ARTHUR J. VORWALD and WILLIAM E. ADAMS (introduced by Esmond R. Long), University of Chicago.

Since one of us (Adams) in 1930 produced massive atelectasis of lung tissue by bronchial stenosis in dogs, this form of treatment was undertaken in cases of pulmonary tuberculosis produced experimentally in dogs.

Massive atelectasis of two or more lobes was produced from two to six weeks subsequent to infection, by cauterizing the mucosa of the corresponding bronchi

with silver nitrate. Most of the animals died or were put to death by the end of four months. The period of infection and the degree of stenosis and subsequent atelectasis of the lung determined the amount of tuberculous tissue that developed. In those lobes in which incomplete stenosis of the bronchial lumen without collapse resulted, there was less tuberculous involvement than in other inflated lobes. In a few animals atelectasis was not attained, either because the animal died before the time required for complete stenosis of the bronchus, or because of physiologic anastomosis between the lobe in which the stem bronchus was stenosed and the normally inflated lobes. In the majority of animals, however, the desired massive atelectasis was obtained. The lobes so collapsed revealed either total absence or decided decrease both in the number of stainable tubercle bacilli and in the tissue reaction to them, whereas the control inflated lobes, in the same animal, contained many tubercles varying in diameter from 1 mm. to 1 cm. In many cases, the inflated lobes contained tubercles with massive caseation and fairly extensive cavitation. Tubercle bacilli in these inflated lobes were demonstrable in countless numbers, particularly in those animals in which the reaction had resulted in caseation and cavitation.

In general, the macroscopic and microscopic observations at autopsy indicate that this method of bronchial stenosis accompanied by permanent massive atelectasis is of unmistakable value in the treatment of experimental pulmonary tuberculosis, when the tuberculous process is concentrated in one or two lobes.

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, May 9, 1932*

R. H. JAFFÉ, *President, in the Chair*

### THREE DIFFERENT TYPES OF TUMORS ARISING FROM THE INFUNDIBULAR RESTS OF THE CRANIOPHARYNGEAL DUCT. H. A. MCKINLEY and ARTHUR WEIL.

At the anterior surface of the infundibulum, islands of squamous epithelium are found, an upper and a lower group, which are separated by the pars tuberalis. The latter has been described by Tilney in animals and by Atwell in human embryos as a sheathlike layer of glandular epithelium. Both types are considered rests of the craniopharyngeal duct. Tumors arising from these groups of cells are adamantinomas, simple squamous epithelial papillary cysts (Duffy) and more complicated teratomas.

Most tumors of the first group seem to be malignant in that they infiltrate the surrounding brain tissue. Most of the squamous epithelial papillary cysts are benign and indent the floor of the third ventricle. An example of each type is described:

1. An adamantinoma in a boy, aged 8 years and 10 months, produced the first symptoms of impairment of vision seven years before death. The tumor had the typical arborization of epithelial cells with an outer layer of ameloblast-like cells and an inner stellate reticulum. It was embedded in a loose stroma of argyrophil connective tissue. Breaking through the floor of the third ventricle, it invaded the walls of this structure and occluded the foramina of Monro. A marked hydrocephalus of the lateral ventricles resulted.

2. A squamous epithelial papillary cyst in a man 32 years old began with impaired vision six months before examination (operated on by Dr. L. Davis). It was cystic and was separated by the thickened pia-arachnoid from the floor of the third ventricle. It consisted of layers of squamous epithelium bordered by cuboidal cells at the outer surface that formed islands of degenerated cells, which were replaced by collagenous connective tissue. Around these islands were palisades of more cylindric cells. No stroma was present.

3. The third tumor was a benign suprasellar growth, which consisted of squamous epithelium and glandular tubules. At the free surface, vacuolated glandular epithelium was found. A loose stroma surrounded the cellular masses. Within the tumor were foci of capillaries and fibroblasts, surrounded by one layer of cuboidal epithelium. This tumor may represent a derivative of the pars tuberalis, because it contained glandular elements together with squamous epithelium similar to the structures described by Tilney and Atwell.

#### LYMPHOGRANULOMA INGUINALE. I. PILOT and L. AMTMAN.

Lymphogranuloma inguinale has been reported as climatic or tropical bubo. The disease is prevalent in Europe and in the United States. By means of a specific skin test (Frei test) many cases previously called inguinal adenitis of possible tuberculous, syphilitic or chancroid origin are now grouped under this clinical entity. Cases have been reported from the United States, but the diagnosis was largely based on the clinical symptoms and microscopic sections. Our three cases, all occurring in men, are the first to be reported from Chicago in which the diagnosis was verified by cutaneous reactions with diagnostic material sent by Frei. This material is the heated exudate aspirated from the glands.

Apparently venereal, the etiology is obscure. Investigations point to a filtrable agent or virus that localizes in the regional lymph glands. Transmission is best obtained in monkeys by intracerebral injection or more recently by inoculation in the prepuce. We were unable to obtain transmission with the pus of two patients, which was inoculated subcutaneously into guinea-pigs.

The inguinal glands enlarge, the capsule thickens, and often the glands become fixed owing to peri-adenitis. Liquefaction occurs, and a thin purulent material may rupture through the skin, with resultant multiple fistulas.

Microscopically, the appearance is not absolutely characteristic. There is diffuse hyperplasia with a distinct tendency to fibrosis. Many plasma cells appear. The softened regions usually contain many polymorphonuclear leukocytes. Giant cells are present occasionally. Smears, stains, cultures and guinea-pig inoculation reveal no tubercle bacilli.

The first patient had been in Nicaragua for several months and noted the adenitis five weeks after returning to the United States. With the enlarging glands, chills and fever developed. The condition was bilateral, and on the left side the glands became adherent to the overlying skin. Thin pus was aspirated, which yielded only *Staphylococcus albus*. A gland was removed, which on section appeared grayish red and revealed small foci of suppuration from 2 to 5 mm. in diameter. Microscopically, the appearance was that of chronic lymphadenitis and peri-adenitis with multiple foci of liquefaction necrosis.

The second patient had not been out of Chicago for two years. A mass was noted as slowly developing in the left groin. A gland was removed, which was soft and grayish red and contained small abscesses. Microscopically, numerous plasma cells and occasional giant cells were observed. Smears and cultures did not reveal organisms. The Frei antigen gave a strong positive cutaneous reaction in both of these patients.

The third patient had been in Chicago for five years. He had enlarged glands and numerous fistulas discharging a thin, gray, sterile exudate. Antigens prepared for the second patient from his own gland, as well as one prepared by Frei in one of his cases, gave positive reactions.

#### DISCUSSION

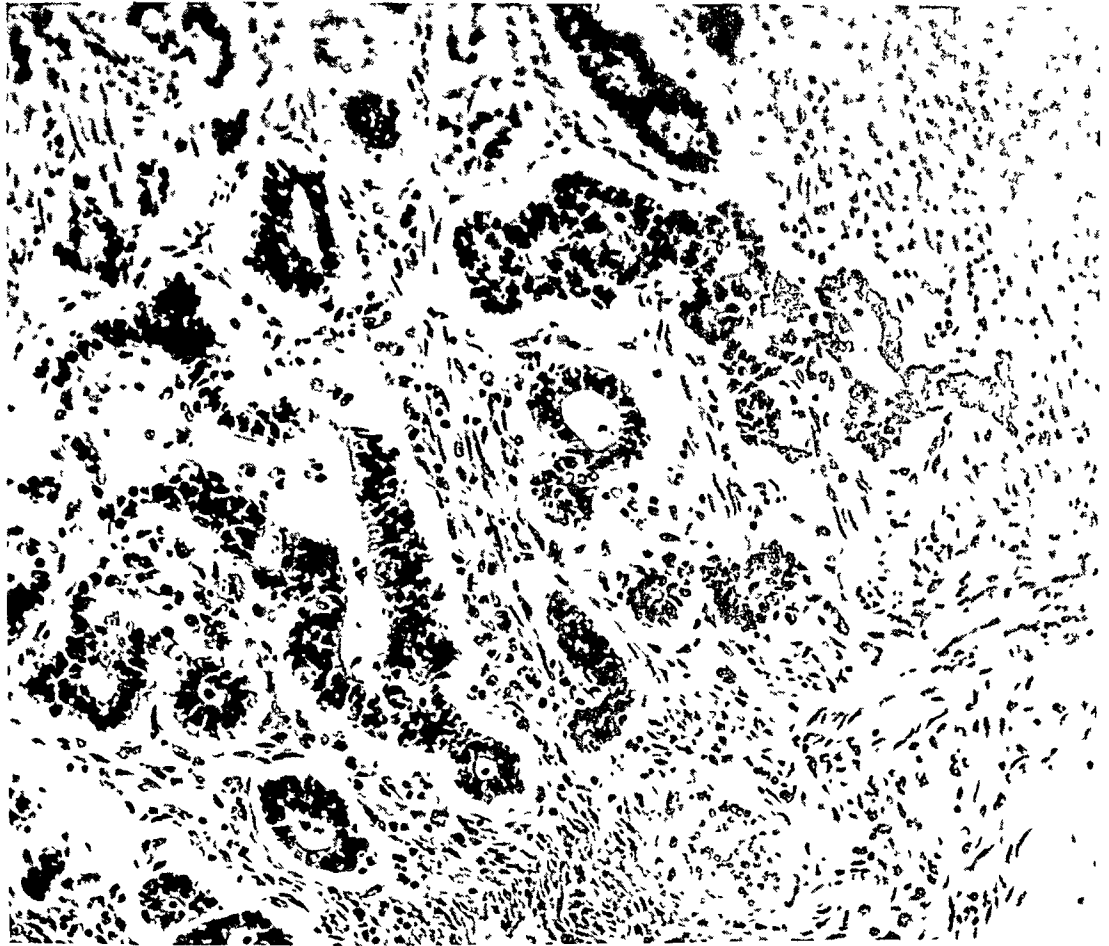
M. FERNAN-NUNEZ: I saw two patients in Nashville, Tenn., who were clinically identical with others seen in the tropics and subtropical regions where this disease is prevalent.

G. W. SCHELM: A patient in North Chicago had similar lesions with ulcerations of the thighs.

R. H. JAFFÉ: The term used for this disease is unsuitable, and some other more appropriate name should be found.

CARCINOMA OF THE STOMACH IN A CHILD AGED THREE YEARS. CORNELIUS S. HAGERTY and STANLEY GIBSON.

Carcinoma of the stomach occurs so rarely in children that there are only six published records of its occurrence in persons aged less than 10 years. Some doubt exists in regard to two of these descriptions because of the uncertainty that the carcinoma was primary or secondary in the stomach. Widerhofer (*Jahrb. f. Kinderh.* 2:194, 1859) recorded multiple nodules of carcinoma in the gastric mucosa, intestines, gallbladder, urinary bladder and pericardium of an infant aged 15 days. He did not describe the origin of the primary tumor



Photomicrograph illustrating the carcinoma tissues in the wall of the stomach

Kaulich (Osler, W., and McCrae, Thomas Cancer of the Stomach, Philadelphia, P. Blakiston's Son & Co., 1900, p. 16) in 1864 described a carcinoma of the stomach in a child aged 1½ years. Cullingsworth (*Brit. M. J.* 2:255, 1877) reported an ulcerated cylindric cell carcinoma of the pylorus of the stomach of an infant. Kuhn (cited by Williams, W. R.: *Lancet* 1:1259, 1897) in 1866 recorded a probably congenital adenocarcinoma of the stomach. Ashby and Wright (*The Diseases of Children*, ed. 4, New York, Longmans, Green & Co., 1900, p. 113) described pea-sized nodules of carcinoma in the stomach and duodenum of a child aged 8 years. Karl (*Deutsche med. Wchnschr.* 41:373, 1915) reported a tumor excised from the stomach of a child aged 9 years, which histologically was a primary carcinoma.

The clinical diagnosis of carcinoma of the stomach in infants and young children is difficult because the disease is unusual at this age of life and is rapidly fatal. The prominent symptoms are loss of appetite, anemia, vomiting, eructations and enlarged abdomen. Pain is rare, and emaciation is usually absent because of the rapid growth of the tumor.

A white girl, aged 3, entered the pediatric service at St. Luke's Hospital, Chicago, Jan. 26, 1932, because of loss of appetite and anemia for three months and an appreciable enlargement of the abdomen for four days. There was a large, nodular, tender mass in the right side of the abdomen, and the feces contained occult blood. The erythrocyte count was 1,110,000; the leukocyte count, 21,350; the hemoglobin was 12 per cent. An exploratory operation January 30 by Dr. S. W. McArthur disclosed metastatic tumor nodules in the liver. The primary tumor was not found. Death occurred on Feb. 2, 1932.

The essential results of the autopsy (Graham A. Kernwein) were: primary ulcerated carcinoma of the cardia of the stomach (perforated); multiple metastases to the liver; marked anemia; acute general fibrinous peritonitis.

The abdomen was markedly distended and contained 270 Gm. of a green-yellow serofibrinous exudate. With the stomach opened along the greater curvature, the lining of the cardia was found replaced by an annular, ulcerated tumor 12 cm. long and 6 cm. wide. The upper level of the ulcer reached the esophagus, and it extended on both the anterior and posterior walls for 6 cm. The base of the ulcer was firm, granular, friable and irregularly pitted. There was a small crescentic perforation of the gastric wall 4 cm. from the upper edge of the ulcer and near the lesser curvature. The edges of the growth were firm, undermined and raised, and averaged 1.2 cm. in width. The surface of the elevated edge was continuous with the pale, gray, edematous mucosa of the stomach. The liver weighed 800 Gm. Under the capsule were many protruding nodules of tumor tissue, and practically two thirds of both lobes consisted of circumscribed nodules of soft, friable, yellow-gray tissue mottled with hemorrhages. The liver tissue otherwise had marked cloudy swelling and fatty changes.

Sections of the tissues from the margin of the gastric tumor showed that the mucosa ended abruptly at the margin of the ulcerated growth and the overhanging ledge. The submucosa and muscularis were invaded by cords and masses of epithelium, differentiated slightly into glandular structures. An abundant connective tissue stroma supported these cells. The metastases in the liver had essentially the same structures.

*Summary.*—There seem to be only six recorded accounts of primary carcinoma of the stomach in children aged 10 or less. A glandular carcinoma of the cardia of the stomach in a child aged 3 is reported. Vague symptoms of the carcinoma appeared from three to four months before death.

#### DISCUSSION

R. H. JAFFÉ: Teratomas may arise in the stomach. I wonder if this began as such a growth and with proliferation of the epithelium.

#### PONTILE HEMORRHAGE IN YOUTH. E. C. PIETTE and E. F. TRAUT.

A woman, aged 25, with nephritis and hypertension for two years, poor vision and headaches, suddenly became unconscious. The systolic blood pressure was 200 mm. of mercury; the diastolic, 100. The urine contained large quantities of albumin and many casts. The spinal fluid was dark red with blood. Death occurred four hours after the onset of coma.

A pontile hemorrhage 4 by 4 by 3 cm. had destroyed almost the entire brain substance below the aqueductus from the mammillary body to the medulla. The floor of the fourth ventricle was lifted up by the hemorrhage, and its cavity was obliterated. The hemorrhage extended laterally into the brachia. Therefore practically all the nuclei of the fifth, sixth, seventh and eighth nerves and the frontal parts of the vagus, accessorius and hypoglossus were more or less completely

destroyed. The hemorrhagic area was dry and crumbling; its outlines were irregular and did not correspond to the distribution of an artery. One branch of one of the arteria medianae pontis located in the center of the hemorrhage appeared torn and possibly represented the point from which the hemorrhage originated.

The kidneys were small, each weighing about 100 Gm. Their capsules were adherent. The cortices were narrow. The heart had marked concentric hypertrophy. It weighed about 350 Gm.

Microscopic examination of various cerebral vessels of larger caliber showed only moderate sclerotic changes, evidenced by thickening of the elastica interna. Detailed examination of various parts of the brain substance near and within the hemorrhagic area showed that a few isolated arterioles were markedly narrowed. Their walls were hyalinized.

Microscopically, the kidneys showed advanced subacute and chronic changes. There were some foci of lymphocytic and plasma cell infiltration, increase in fibrous tissue and calcification of tubular epithelium. The arterioles had few if any sclerotic changes.

#### DISCUSSION

R. H. JAFFÉ: Probably this hemorrhage had a uremic basis.



## Book Reviews

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**Roentgenologic Studies of Egyptian' and Peruvian Mummies.** By Roy L. Moodie, Paleopathologist to the Wellcome Historical Museum, London. Berthold Laufer, Curator of Anthropology, Editor. *Anthropology Memoirs, Field Museum of Natural History Founded by Marshall Field, 1893. Volume 3.* Paper. Price, \$5. Pp. 66, with 76 plates. Chicago: Field Museum of Natural History, 1931.

In 1926, the Field Museum installed an x-ray equipment for the study of specimens in its collection. It was one of the leaders among museums of natural history, if not the first, to make systematic use of this method of research. Some of the results are shown in the present volume. It is superbly illustrated with photogravure reproductions of the admirable roentgenograms made by Miss Anna R. Bolan, of the Field Museum staff. The contents of mummy wrappings and cases are revealed in a striking and dramatic fashion. The roentgenograms showing the skeletons of a little boy and his sister side by side, bodies embalmed and wrapped from which parts are missing, which probably were lost by the embalmers, and the supposed mummy of a cat, all of which was faked except the cranium, must interest any observer. Some specimens are illustrated by photographs. The text by Roy L. Moodie discusses the processes of mummification and the diseases of ancient Egypt and Peru. Among the pathologic conditions described are arthritis deformans, pyorrhea alveolaris, dental caries, impacted teeth, injuries of the skull, trephining of Peruvian skulls and symmetrical osteoporosis. Interesting are the roentgenogram of an Egyptian mummy with a tortuous calcified artery, a probable hydrocephalus in a Peruvian child, a roentgenogram of a Peruvian skull with a tumor, apparently osteosarcoma, and photographs of Peruvian skulls with osteomas of the external auditory meatus. A case of symmetrical osteoporosis of the frontal bone of a child in which trephining had been done is important, as it indicates that the disease of the bone may have been the motive for the operation. The reason for the operation is not apparent in most cases of prehistoric trephining.

**Neoplasms of Domesticated Animals.** By William H. Feldman, D.V.M., M.D., Division of Experimental Surgery and Pathology, the Mayo Foundation, Rochester, Minn. With a Foreword by Charles H. Mayo, M.D. Cloth. Price, \$6 net. Pp. 410, with 193 illustrations. Philadelphia: W. B. Saunders Company, 1932.

The prominence of experimental work with cancer in animals during the past thirty years has given the tumors of animals a large importance in the study of malignant disease. Previously there has been available no book systematically discussing the tumors of lower animals, a want now partly met by Feldman's contribution. Primarily written to supply the needs of students and practitioners of veterinary medicine, the book deals chiefly with tumors in the animals handled by the veterinarian, and discusses them largely from the standpoint of the general pathology of the new growths, with numerous excellent illustrations that demonstrate the identity of the neoplasms of animals with those seen in man. The work seems well adapted for use by the veterinarian, as intended. It will also be of use to the general pathologist, who often meets with tumors in animals from one source or another. The investigator of the problem of tumors will find the bibliography useful, and it will often serve as a source of useful information concerning comparative pathology, although it is by no means a complete guide to the widely scattered literature of this field. It serves to emphasize the serious failure of the Bureau of Animal Industry in neglecting to have recorded in avail-

able and adequate manner the innumerable observations of its inspectors on the tumors found in the abattoirs of the United States. Here is a vast and invaluable material that has been wasted.

**Histopathology of the Central Nervous System. An Introduction by Means of Typical Microphotographs and a Short Text.** By Prof. Dr. L. Bouman, Utrecht, and Prof. Dr. S. T. Bok, Leiden. Price, 25 florins. Pp. 37, with 212 figures on 53 plates. Utrecht: A. Oosthoek's Publishing Company, 1932.

The book is an atlas of 212 excellent photographs printed on glossy, photographic paper and accompanied by a text of 37 pages. The photographs, or rather photomicrographs, are for the most part splendidly done and, though not in colors, convey an excellent idea of the subjects treated. It is not a systematic or detailed book on neuropathology, but a photographic illustration of the most important phases of normal and pathologic histology of the central nervous system. The peripheral nerves, malformations of the nervous system and tumors were not included. Syringomyelia was excluded from the review, as the authors consider it a tumor formation. The Spanish staining methods and classification of the glia elements have been given careful consideration, but microglia is classified by them not as connective, but as neuroglia, tissue. Because of the remarkably clear and concise manner with which the fundamental topics of neuropathology have been dealt, the book will prove extremely valuable and useful to the busy general pathologist and to the clinician.

**Medical Aspects of Old Age: Being a Revised and Enlarged Edition of the Linacre Lecture, 1922.** By Sir Humphry Rolleston, Bart., G.C.V.O., K.C.B., M.D., Regius Professor of Physic in the University of Cambridge. Second edition. Cloth. Price, \$3. Pp. 205, with 7 portraits. New York: The Macmillan Company, 1932.

There are ten chapters in the book: duration of life, onset of old age, factors influencing longevity, causes of old age, normal structural changes in old age, the description of old age in the twelfth chapter of Ecclesiastes, distinction between healthy and morbid old age, diseases of old age, old age and carcinoma. There are an index of persons and one of subjects. The seven illustrations show persons reputed to have lived from one hundred to one hundred and eighty-five years. The style is clear and pleasing with occasional touches of gentle humor. An air of quiet and thoughtful consideration prevails. Except for incidental references, medical treatment in old age has not been considered.

**Tuberkulose als Schicksal. Eine Sammlung pathographischer Skizzen von Calvin bis Klabund 1509-1928.** Von Dr. Erich Ebstein. Mit einer Einführung von Prof. Dr. Georg B. Gruber. Pp. 191, with 8 illustrations. Price, 6.50 marks. Stuttgart: Ferdinand Enke, 1932.

This book was published after the death of the author. It contains about fifty short pathographic sketches of remarkable persons who lived in the period from 1509 to 1928. The purpose of these sketches was to furnish a basis for a closer study of the mind in pulmonary tuberculosis, especially of gifted persons, but this study was not completed when the author died, and in its place is an introductory chapter on the psychology of tuberculosis by Georg B. Gruber. The book contains many interesting data and references. It will be of special interest to students of tuberculosis and of biography.

## Books Received

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ABHANDLUNGEN AUF DEM GEBIETE DER ALLGEMEINEN PATHOLOGIE UND PATHOLOGISCHEN ANATOMIE. Zweiter Band. Mitteilungen des Pathologischen und Histologischen Institutes der KGL. Ung. Franz Josef Universität, Szeged, Ungarn. Herausgegeben von Dr. Josef Baló, O. O. Universitätsprofessor Direktor des Institutes. Szeged: 1931.

UEBER WACHSTUM UND RÜCKGANG; UEBER STANDARDISIERUNG, INDIVIDUALISIERUNG UND BAULICHE INDIVIDUALTYPEN IM LAUFE DES NORMALEN POSTFÖTALLEBENS, KONSTITUTIONSANATOMISCHE STUDIEN AN KANINCHEN. Von J. Aug. Hammar, Professor Emer. an der Universität Uppsala. Pp. 540, mit 132 Tabellen, 48 Text Abbildungen und 10 Abbildungen auf Tafel I-V. Leipzig: Akademische Verlagsgesellschaft M.B.H., 1932.

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1930. By Major L. A. P. Anderson, I.M.S. Offg. Director Haffkine Institute. Price, 8 pence 6 annas. Pp. 76. Bombay: Government Central Press, 1932.

SPECIAL CYTOLOGY: THE FORMS AND FUNCTIONS OF THE CELL IN HEALTH AND DISEASE. A Textbook for Students of Biology and Medicine. Edited by Edmund V. Cowdry, Washington University, St. Louis, Mo. Edition 2. Three Volumes. Price, \$30. Pp. 1,838, with 757 illustrations. New York: Paul B. Hoeber, Inc., 1932.

THE HEART RATE. By Ernst P. Boas, M.D., Associate Physician, Mount Sinai Hospital, New York, and Ernst F. Goldschmidt, Ph.D., Research Fellow (1930-31), Department of Surgery, Yale University School of Medicine. Price, \$3.50. Pp. 166, with 68 figures. Springfield, Ill.: Charles C. Thomas, 1932.

REPORT OF THE LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADELPHIA. By Herbert Fox, M.D., Pathologist, 1932.

THE SIGN OF BABINSKI: A STUDY OF THE EVOLUTION OF CORTICAL DOMINANCE IN PRIMATES. By John F. Fulton, Sterling Professor of Physiology in the Yale University School of Medicine, and Allen D. Keller, Professor of Physiology and Pharmacology in the School of Medicine, University of Alabama. Price, cloth, \$5. Pp. 166, with 66 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

## CULTURES OF LEUKEMIC BLOOD LEUKOCYTES

MILA PIERCE, M.D.

CHICAGO

The number of existing theories of the formation of blood is sufficient evidence that the understanding of the origin and the fate of blood cells is still confused. During the past fifteen years several investigators have employed the method of tissue culture for studying the behavior, origin and development potencies of leukocytes in vitro. The results have been gratifying, since the cells could thus be observed while isolated from such complicating elements as blood vessel endothelium and connective tissue cells. •

The following study of leukocytes in leukemic blood cultures was undertaken with the purpose of investigating several points of interest to both hematologist and clinician:

1. Do leukemic blood cells in culture behave differently from normal leukocytes?
2. Does the lymphoblast of acute lymphatic leukemia in culture differ in its behavior from the myeloblast of acute myeloid leukemia?
3. Do the lymphocytes of chronic lymphatic leukemia react like those of acute lymphatic leukemia?
4. What are the developmental potencies of the small lymphocyte?
5. Can the observations of Timofejewsky and Benewolenskaja (1929) be corroborated; i. e., that the myeloblast (hemocytoblast) of acute myeloid leukemia is capable of producing monocytes, giant cells, fibroblast-like cells, plasma cells and cells of the erythroblastic and granulocytic series?
6. Is a monocyte-like cell found as a transitional form in both lymphatic and myeloid leukemia, and what further light can be thrown on the origin of the monocyte?
7. Are cultures of leukocytes of the large cell ("blast") type of leukemia of aid in facilitating the clinical diagnosis?
8. Which of the theories of the formation of blood are substantiated by the observations on behavior of leukemic leukocytes in vitro?

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From the Otho S. A. Sprague Memorial Institute, the Children's Memorial Hospital of Chicago, and the Department of Anatomy of the University of Chicago.

This investigation was aided by a grant from the Rockefeller Foundation to the Division of Biological Sciences of the University of Chicago.

## LITERATURE

Normal blood leukocytes have been cultured with modern tissue culture technic during the past fifteen years by a number of investigators, most of the observations being made on blood of experimental animals. In 1915, Awrorow and Timofejewsky reported the results of cultivation of the blood leukocytes of the normal rabbit, dog and guinea-pig, in which they showed that lymphocytes and monocytes are capable of differentiating into polyblast-like and fibroblast-like cells.

Carrel and Ebeling (1922) found that pure cultures of ameboid phagocytic elements (which they called large mononuclear leukocytes) developed in cultures of chick blood; they were uncertain of the origin of these cells. They noted that the lymphocytes spontaneously disappeared after the first week; they thought that it was possible that these cells were transformed into the larger forms.

M. A. Lewis (1926), who used chick, rabbit, frog and human blood cultures, also reported observation of the development of phagocytes and later fibroblast-like cells. Lewis did not derive these elements from lymphocytes; but concluded that they had developed from the monocytes of the blood.

A. Maximow (1925) noted that in cultures of blood leukocytes inoculated with tubercle bacilli, both lymphocytes and monocytes hypertrophied into macrophages and epithelioid cells.

In 1928, Maximow published a comprehensive study in which he reported observations on all the different types of leukocytes in the chick, guinea-pig, rat, rabbit and monkey in supravitaly stained living cultures and fixed and stained cultures. He pointed out the following significant facts: 1. The granular leukocytes began to degenerate shortly after explantation and at the end of forty-eight hours had disintegrated; the lymphocytes and monocytes were transformed by change of individual cells in the course of a few hours into large ameboid, phagocytic, dye-storing elements, which were identical in origin, structure and function with most of the polyblasts or mononuclear exudate cells as found in inflamed tissue. Mitoses occurred in the hypertrophying lymphocytes and monocytes, but were rare. 2. In this transformation into polyblasts, the lymphocytes passed through a monocyte-like stage. 3. The polyblasts developed into fibroblast-like cells and later into typical fibroblasts. In his last work (1928, 1929) he reported the development of reticular and collagenous fibers in 25 day old cultures of blood leukocytes. He concluded that the phagocytic and fibroblastic potencies of the lymphocytes of the blood were conclusively demonstrated.

Awrorow and Timofejewsky (1914) were the first to culture human leukocytes in cases of myelogenous leukemia. They observed that an intense migration of the explanted cells into the medium occurred; the

mature granular elements died out after a few days; the "myeloblasts," which they did not distinguish from lymphocytes and large mononuclear cells, rapidly enlarged and developed into phagocytes, and later into spindle cells, fibroblast-like cells and giant cells. Some of the myeloblasts were thought to change into myelocytes.

Timofejewsky and Benewolenskaja (1926-1929) reported a detailed study of the leukocytes in three chronic lymphatic, one subacute lymphatic, one atypical lymphatic, and two myeloid leukemias. The cultures were studied in the living condition and in sections. They found, in cultures of normal blood and in that of lymphatic leukemia, lymphocytes developing into monocyte-like cells, macrophages and fibroblast-like cells. The most noteworthy results were seen in their cases of myeloid leukemia, in which the majority of the explanted cells were classified as hemocytoblasts, i. e., myeloblasts. They observed the development of specific granules in the myeloblasts, and also the transformation of myeloblasts into erythroblasts. The height of granulopoiesis was reached in twenty-four hours, and that of erythropoiesis, in forty-eight hours. Both myelocytes and erythroblasts degenerated in from three to five days. On the basis of their investigations these observers concluded that:

1. Lymphocytes of normal blood and lymphatic leukemia can develop into macrophages, plasma cells, epithelioid cells, multinuclear giant cells and fibroblast-like cells.

2. Hemocytoblasts of acute myeloid leukemia can develop in vitro into macrophages, plasma cells, epithelioid cells, multinuclear giant cells, myelocytes, erythroblasts and normoblasts.

3. The lymphocytes of normal blood and of lymphatic leukemia and the hemocytoblasts of myeloid leukemia pass through a monocyte-like stage in the course of their development into macrophages.

Hirschfeld (1927) cultured the leukocytes of leukemia in human plasma, using those of three chronic (small cell) lymphatic, two acute myeloblastic and two myeloid leukemias; he reported conditions after twenty-four, forty-eight and seventy-two hours (in one case observations were made at six hours). From these studies he concluded that the cells of myeloid and lymphoid leukemia develop into fibroblast-like cells and macrophages, but that these are derived from the myeloblasts in the myeloid cases, and from monocytes in the lymphoid cases. Since no mitotic figures were seen, and since the percentage of monocytes in these lymphatic leukemias was admittedly small, Bloom (1928) and Timofejewsky and Benewolenskaja (1929) did not believe that the macrophages in Hirschfeld's experiments could have developed only from monocytes.

Hirschfeld and Klee-Rawidowicz (1928) cultured the blood cells in a case of lymphatic leukemia in which the blood consisted almost exclu-

sively of lymphocytes with a few monocytes. The lymphocytes were ameboid and formed pseudopodia; the migrating monocytes became greatly enlarged and took on the appearance of reticular cells. They concluded that the monocytes seemed to have increased greatly both in number and size.

Veratti (1928) cultured the leukocytes in two cases of myeloid leukemia and noted the development of cells with the properties of histiocytes, large giant cells and elongated cells. He believed that these cells were developed in normal blood from monocytes and in leukemic blood from monocytes and hemohistioblasts.

Bloom (1927) cultured the lymph from the thoracic duct of rabbits, which contains few cells other than small and large lymphocytes. As the lymphocytes hypertrophied they went through a monocyte-like stage and then developed into polyblasts and fibroblast-like cells. Bergel (1930) obtained similar results in cultures of rabbit lymph.

Parker and Rhoads (1928) reported observations on incubated normal and leukemic leukocytes. They noted that most of the polymorphonuclear neutrophils disintegrated after twenty-four hours, although some lived for a longer time and developed refractile granules. The eosinophils survived longer than the neutrophils and showed no degenerative change. The lymphocytes lived as long as the eosinophils and retained their normal characteristics throughout life; no increase in size of these cells was noted, and they were not phagocytic. However, in the ascitic fluid of a case of chronic lymphatic leukemia, in which most of the cells were typical small lymphocytes and in which there were no monocytes, large phagocytes were observed after the fluid had been incubated for six days. The authors pointed out that this finding bears out the work of Bloom on cultures of lymph.

#### TECHNIC

From 10 to 12 cc. of blood was drawn aseptically from the median basilic vein into a syringe containing 1 cc. of heparin (1:2,500 in 0.85 per cent sodium chloride solution) to prevent coagulation, and transferred to a sterile centrifuge tube. As soon as possible, although in some instances there was an interval of one or one and one-half hours because of the distance of the hospital from the laboratory, the blood was centrifugated at high speed and the plasma pipetted off. The buffy coat was then coagulated by the addition of from 1 to 3 drops of rabbit embryonic extract and undiluted rabbit plasma. After it had been allowed to clot in the incubator, the buffy coat was removed with a glass spoon, placed in a hollow slide containing from 2 to 3 cc. of Tyrode solution, and cut into very small pieces with a pair of fine, sharp scissors. The cultures were made in large hanging-drop slides as follows:

A minim (0.06 cc.) of Tyrode solution was dropped on a large square (40 by 40 mm.) coverslip, and a small round (21 mm.) coverslip was placed over it. A drop of diluted rabbit plasma with Tyrode solution and distilled water in the ratio of 4:2:1 was placed on the round coverslip, a small bit of buffy coat added, and the

drop coagulated by the addition of an equal amount of rabbit embryonic tissue extract diluted with 6 parts of Tyrode solution. After the plasma had coagulated, the coverslip was inverted over the hollow slide, the chamber sealed with petrolatum and a layer of paraffin, and the slides placed in the incubator. This is the Maximow method of tissue culture. From thirty to forty slides were made in each case.

For control material the remainder of the buffy coat was fixed immediately and carried through the same processes of fixation and staining as the cultures.

The cultures on the slides were observed in the living condition, fixed at six hour intervals or less during the first twenty-four hours, and at twelve hour intervals or less after that time. As the most significant changes occurred during the first two days, a special attempt was made to study these early changes in detail. The cultures were fixed in Zenker's solution plus formaldehyde solution, embedded in celloidin, serially sectioned and stained with hematoxylin-eosin-azure II solution.

#### CASE REPORTS AND OBSERVATIONS IN LEUKOCYTIC CULTURES

For the sake of brevity the case histories and necropsy findings are not given in detail, although careful study was made of each case.

CASE 1.—*Acute Lymphatic Leukemia*.—V. H., a girl, aged 4, a patient of Dr. Joseph Brennemann at the Children's Memorial Hospital over a period of nine months, during this time had three exacerbations of fever, lymphocytosis followed by leukopenia, splenomegaly and moderate lymphadenopathy. The remission after the first attack lasted three months, and after the second, six weeks. The terminal picture was that of acute lymphatic leukemia with the classic syndrome of lymphadenopathy, great enlargement of the liver and spleen, hemorrhages from the mucous membranes, necrotic areas in the mouth and throat and a lymphocytosis of 33,000. Within the twenty-four hours before death, large amounts of urates were excreted in the urine, the liver and spleen decreased markedly in size, and the total white count dropped to 1,000.

At necropsy, a dense lymphocytic infiltration of the bone marrow was found, with a less marked infiltration of the liver and kidney. The spleen and lymph nodes were moderately enlarged, and the germinal centers were actively proliferative. It was felt by three pathologists who examined the sections that the process was of a leukemic nature, although the extent of infiltration was less than that which is usually seen in acute lymphatic leukemia.

Observations in Leukocyte Cultures: The blood cell counts on the day of culture (May 11, 1931) were as follows: red, 3,600,000, with hemoglobin 45 per cent (Dare); white, 28,450; differential (Wright's stain), 2 per cent band-shaped and 1 per cent segmented polymorphonuclear leukocytes, 94 per cent small to medium-sized lymphocytes, 2 per cent leukocytoid lymphocytes, 0.3 per cent eosinophils, 0.3 per cent basophils, 1 per cent erythroblasts, and 10 per cent fragmentary cells.

The nucleus of the lymphocytes was oval, with a slight indentation on one or occasionally on both sides. The chromatin pattern was coarse and the nuclear membrane heavy. In the larger lymphocytes one or two nucleoli were seen. The nucleus almost completely filled the light blue cytoplasm, which contained a varying number of azure granules; in a few cells the cytoplasm was finely vacuolar. The lymphocytes varied in size from that of an erythrocyte to half again as large, and because of the great number of intermediate sizes it was almost impossible to separate the small from the medium-sized lymphocytes.



Control sections made from the bits of buffy coat remaining after the cultures were explanted were made up of a dense collection of erythrocytes, among which leukocytes were liberally distributed. In some areas the leukocytes formed dense clusters, and in others they were scattered sparsely among the red cells. The great majority of the leukocytes were small lymphocytes with a diameter equal to that of an erythrocyte, although a few were slightly larger. They had an oval nucleus with one or two indentations of the heavy nuclear membrane, a coarse chromatin pattern and basophilic, nongranular cytoplasm. The absence of cells of the monocyte type was noteworthy. Very few polymorphonuclear neutrophils and eosinophils were scattered in the control material. A few ameboid lymphocytes and polymorphonuclear neutrophils were seen at the periphery.

The control culture, fixed immediately after explantation without being incubated and approximately two hours after the blood had been drawn for culture, was made up of the same types of cells, consisting chiefly of small lymphocytes unevenly distributed among erythrocytes. In the less densely crowded areas, the cells appeared larger, and more of the medium-sized lymphocytes were seen. At the periphery of the tissue a few ameboid lymphocytes were found, having an elongated, oval shape, and plump, deeply indented nuclei. A few monocytoid cells were seen in this region of the explant. There were relatively few granular cells, although a very few polymorphonuclear neutrophils and a larger number of eosinophils were seen. Most of the eosinophils had two or three lobes to their nuclei, but a rare eosinophilic myelocyte was present.

After two hours, a few lymphocytes had migrated from the periphery of the explant; those at the outer edge of the zone of migration were elongated, irregular cells, each with a relatively large amount of nongranular cytoplasm and an oval nucleus; those nearer the explant were round or oval; each had more cytoplasm than similar cells within the explant, and an oval, slightly indented nucleus. Within the explant there had been definite hypertrophy of some cells, although in the great majority there had been little change. The hypertrophied cells each had an increased amount of cytoplasm and a large plump nucleus containing one or more nucleoli and showing an irregular nuclear membrane. There were several monocytes with horseshoe-shaped nuclei. More polymorphonuclear neutrophils were seen in this culture than in either the control tissue or the culture. Eosinophils were frequent.

After five hours, there had been little migration of the cells from the periphery of the explant, but there were ameboid forms within the explant. There were many medium-sized lymphocytes and an occasional macrophage with an elongated, crescent-shaped nucleus and nongranular cytoplasm containing vacuoles and cellular debris. There were many polymorphonuclear cells, in some of which the nuclei were pyknotic and broken. A few eosinophils were present. No mitotic figures were seen.

After twelve hours, most of the polymorphonuclear cells and many of the lymphocytes had pyknotic nuclei and degeneration vacuoles in the cytoplasm. While there were many unchanged small lymphocytes, the majority had increased further in size. Frequent monocyte-like and elongated cells, as well as dumb-bell-shaped or ameboid lymphocytes were seen. An occasional large phagocytic, nongranular cell contained ingested debris. In the less crowded areas a few large lymphocytes were found. No mitosis was observed. Frequent eosinophilic leukocytes and myelocytes were scattered throughout the section.

After twenty-one hours, there was a wide zone of migration about the explant, made up of ameboid mononuclear and very few polymorphonuclear cells. The majority of the cells were rounded and elongated lymphocytes with bizarre

nuclear forms, i. e., dumb-bell-shaped, rounded, triangular or knobbed. A few of these cells had the typical nucleus of a polyblast.

Within the explant, among the mass of medium-sized lymphocytes and degenerating cells, many larger lymphocytes (which corresponded with the larger, medium-sized lymphocytes of the lymphatic tissue) were found, each with a large, round, vesicular nucleus, two or three prominent acidophil nucleoli, and a thin rim of nongranular cytoplasm. There were also many medium-sized lymphocytes—larger than the explanted small ones—which had rounded cell outlines, but the nuclei of which were deeply indented on all sides. Many monocyte-like cells in which the nucleus-plasma relationship was equal were present.

After twenty-eight hours, large numbers of dying cells were present in the cultures. Those cells that were living were all larger than the ones originally explanted. There were many very large lymphocytes, having large round nuclei and deeply basophilic cytoplasm, and also large numbers of phagocytic cells containing ingested debris with irregular or crescent-shaped nuclei. Frequent monocyte-like cells, as large as a medium-sized lymphocyte, and a few cells typical of the large mononuclear cells were seen. Eosinophils were rare. The medium-sized lymphocytes had the deeply lobulated nuclei described. No mitotic figures were seen.

After forty-five hours, there had been a striking hypertrophy of the mass of living cells into large lymphocytes, monocyte-like cells and hemocytoblasts. Islands of living cells in which there were no mitotic figures were scattered among the less cellular areas in which there were many degenerating cells. These islands were made up of the following cell types: (1) a few small lymphocytes, most of which had an irregularly indented nucleus; (2) somewhat larger lymphocytes having the same irregular nucleus and a thin band of basophilic cytoplasm; (3) cells of the same size as these larger (medium-sized) lymphocytes in which the nucleus was horseshoe-shaped or kidney-shaped, and in which there was a large amount of cytoplasm (these may be called monocytoïd lymphocytes); (4) somewhat larger cells with a horseshoe-shaped nucleus and a large quantity of slightly basophilic cytoplasm, which were monocytes; (5) very large lymphocytes with a round nucleus, two or three nucleoli and a narrow rim of basophilic cytoplasm; a few of the latter were very large lymphocytes with a large, kidney-shaped nucleus containing heavy chromatin particles, two or three acidophil nucleoli and a distinct nuclear membrane surrounded by a basophilic cytoplasm in which an attraction sphere might be seen opposite the indentation of the nucleus (see plate). These cells were identical in appearance with the hemocytoblasts of Maximow, Jolly (1923) and others. Scattered through the culture were many large lymphocytes and monocyte-like cells. There were also many single hemocytoblasts standing alone among grouped dying cells. The number of monocytoïd cells in the cultures was strikingly high. Frequent eosinophilic leukocytes, but no neutrophils, were present throughout the culture. Occasional phagocytic cells were seen.

After fifty-three hours, the same cell types as described in the forty-five hour cultures were to be found. In addition, there were many large monocyte-like cells in which the nucleus was vesicular, containing a large amount of nuclear sap and widely separated chromatin particles. The number of phagocytic cells had increased. There were a few cells with a crescent-shaped nucleus and a vacuolated cytoplasm in which granules that appeared to be eosinophilic were arranged at the periphery of the cytoplasm. These were probably macrophages with ingested eosinophilic granules. A few eosinophils with pyknotic nuclei were scattered throughout the culture.

After seventy-two hours, the cultures showed little change. Many islands of living cells were grouped among the cellular debris. The number of monocyte-like

cells had increased; they were larger than in the earlier cultures. Some of the largest ones contained ingested material and were now obvious macrophages. Although many hemocytoblasts were present, they were less frequent than in the forty-five hour cultures. There were many elongated, fibroblast-like cells among the monocyte-like cells, each with a pale, vesicular, oval nucleus, prominent acidophil nucleoli and a polyhedral cytoplasm. An occasional very large cell with two large, vesicular nuclei with heavy chromatin particles, acidophil nucleoli and a deeply basophilic cytoplasm was seen. Mitotic figures were absent.

In the four and five day cultures, most of the cells had degenerated, only a few phagocytic cells and monocyte-like cells remaining.

In summary of the observations in the cultures of the leukocytes in this case of atypical lymphatic leukemia, in which the total white cell count on the day of culture was 28,450, with 94 per cent smaller lymphocytes, it may be said:

1. The lymphocytes were actively ameboid shortly after explantation.
2. The lymphocytes hypertrophied as early as two hours after explanation.
3. Early in this process of hypertrophy, the cells assumed a monocyte-like appearance.
4. After twenty-eight hours, large numbers of large lymphocytes, monocyte-like cells and a few macrophages were present.
5. After forty-five hours, the living cells were medium-sized lymphocytes, large lymphocytes, monocyte-like cells, large monocytes and hemocytoblasts, and a few eosinophils.
6. In the older cultures, the monocyte-like cells appeared to become epithelioid and the number of phagocytic cells to increase.
7. No evidence of erythropoiesis or of granulopoiesis was found.

The outstanding feature of this case is, then, the development of numerous large lymphocytes (typical hemocytoblasts or basophilic stem cells of Maximow) from the explanted small and medium-sized lymphocytes. In the absence of mitotic figures and in the presence of large numbers of transitional forms between small lymphocytes and the hemocytoblasts, one can only conclude that the small lymphocyte is capable of developing into the large basophilic stem cell which is normally found in the lymphatic tissue, and in the bone marrow.

*CASE 2.—Acute Myeloblastic Leukemia.*—C. P., an Italian, aged 36, was admitted to Cook County Hospital, Nov. 3, 1930, with a history of illness of seven months' duration, the initial symptoms of which were pain in the sacro-iliac region and fatigue. He had received roentgen treatment over the spleen at the Presbyterian Hospital in June, 1930. The acute symptoms began one month before admission, when anorexia, malaise and nausea developed. During the hospital course the picture presented was that of acute leukemia with progressive anemia, leukocytosis with large numbers of blast forms and myelocytes, hemorrhages from the mucous surfaces, generalized lymphadenopathy and splenomegaly. He died on Nov. 26, 1930.

The blood cell counts by the attending intern were as shown in table 1.

The anatomic diagnosis made at necropsy by Dr. R. H. Jaffé was as follows: chronic myelogenous leukemia; petechial hemorrhages in the mucous membranes, lips, skin, epicardium and endocardium, pleura and pulmonary parenchyma; diphtheritic colitis; hyperplasia of the cervical, axillary, inguinal, epitrochlear, peri-aortic, peribiliary and pancreatic lymph nodes; severe anemia of the internal organs.

Observations in Leukocyte Cultures: At the time of withdrawal of blood for the cultures, the cell counts were as follows: red, 1,960,000; white, 48,900; differential, 84 per cent myeloblasts, 1 per cent metamyelocytes, 12 per cent polymorphonuclear neutrophils, 2 per cent basophils, 1 per cent lymphocytes and 1 normoblast per two hundred leukocytes.

As seen in the dry smear stained with May-Grünwald-Giemsa stain, the myeloblasts were round cells varying in size from that slightly larger than an erythrocyte to one twice as large. The large nucleus had an almost imperceptible nuclear membrane and a fine chromatin arrangement with one or two nucleoli, about which

TABLE 1.—*Blood Counts in Case 2: Acute Myeloblastic Leukemia*

Date	Red Cells	Hemoglobin, per Cent	White Cells	Differential, per Cent								Nucleated Red Cells
				Myeloblasts	Premyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclear Neutrophils	Small Lymphocytes	Basophils	Eosinophils	
7/11/30	4,500,000	84	103,000	..	..	31	..	65	1	3	..	..
7/12/30	4,860,000	87	97,000	..	..	31.4	..	47.6	3.3	1.4	0.9	..
11/26/30	1,570,000	50	41,950	81	3	0	1	12	1	2	..	1

there was a heavy condensation of chromatin particles. The separation of chromatin and parachromatin was not distinct. The nucleus was surrounded by a halo of pale blue, finely vacuolated cytoplasm, which was denser at the periphery, so that the cell was sharply outlined by a deep blue rim.

In the control cultures fixed immediately after explantation (about two hours after removal of the blood from the body), most of the cells were lymphoid. The majority were slightly larger in diameter than an erythrocyte. The nucleus was round or oval, contained two or three large particles of chromatin joined by delicate strands, and was sharply demarcated from the encircling narrow rim of basophilic, nongranular cytoplasm by a distinct membrane. At the periphery of the culture were a few ameboid cells. A few polymorphonuclear cells in which the fine neutrophilic granules were readily visible in the cytoplasm were scattered throughout the culture. A rare normoblast was seen in these control sections.

At the end of six hours, active migration was present around the periphery of the explant. At the outer edge of the zone of migration there were many polymorphonuclear leukocytes, the cytoplasm of which was filled with fine, almost imperceptible, reddish-purple granules. The majority of the ameboid cells had the appearances of the exudate mononuclear cells or polyblasts, as described by Maximow (1902-1928); they were characterized by an eccentric, irregular and wrinkled nucleus and irregular, cloudy blue cytoplasm, which assumed a variety of shapes. While most of the polyblasts were rounded, a few were elongated and had long triangular pseudopodia.

Scattered among these polyblasts were ameboid cells that had a single-lobed nucleus gathered at one end of the cell and an irregular, definitely granular cytoplasm. These granules were very slightly larger than those in the polymorphonuclear cells and stained dull red. These cells were recognized ameboid myelocytes. A very rare eosinophilic myelocyte was seen. Within the explant there had been hypertrophy of most of the cells, although those in the more crowded areas remained unchanged. The enlargement had occurred in both the nucleus and the cytoplasm, although the latter was more abundant than in the original cells, and the nucleus had assumed an eccentric position.

After twelve and eighteen hours, the zone of migration was wider and contained a variety of cell forms. The largest number of cells were rounded and elongated polyblasts, polymorphonuclear neutrophils, ameboid neutrophilic myelocytes and an occasional dying cell with a pyknotic nucleus. A few of the ameboid polyblasts contained ingested debris. Scattered among the ameboid cells were numerous<sup>1</sup> neutrophilic myelocytes; in these cells, the nucleus was round or slightly indented, and the cytoplasm contained very fine neutrophilic granules; in many the cytoplasm bulged to one side of the nucleus. Numerous rounded myeloblasts were also present near the edge of the explant. In the zone of migration the outstanding feature was the large number of transitional forms between myeloblasts and ameboid polyblasts. Within the explant at this stage there had been hypertrophy of the majority of the cells; the nuclei were larger and more vesicular, and contained from one to three acidophil nucleoli, and were frequently deeply lobulated; the cytocentrum was prominent in many. A few cells had a relatively large amount of cytoplasm and were morphologically similar to monocytes. A few myelocytes were distinguished. Most of the cells that were not closely packed had assumed a slightly irregular, frequently elongated shape and appeared to be ameboid. No polymorphonuclear neutrophils were found within the explant; apparently these elements had all migrated into the medium. The culture was sparsely dotted with pyknotic nuclei of dying cells. At the edge of the explant, an occasional typical normoblast was seen, and in a few of these the nucleus was in the process of being extruded.

After forty hours, very few polymorphonuclear leukocytes were seen in the zone of migration, and most of those present had pyknotic nuclei. The polyblasts had increased in number and size; there were now many spindle-shaped and polyhedral cells with long cytoplasmic processes. Close to the explant were rounded polyblasts and relatively few neutrophilic myelocytes.

Within the explant were active phagocytes about the size of an erythrocyte; their cytoplasm contained debris, and their nuclei were crowded to one side by the ingested particles. However, most of the cells here were nonphagocytic, had large, round or oval, vesicular nuclei, each occupying about two thirds of the cell, and basophilic nongranular cytoplasm. There were a few mitotic figures within the explant. Scattered throughout the explant were many cells characterized by a deeply pyknotic nucleus and an acidophilic rounded cytoplasm, which resembled normoblasts. However, there was a great variety in the size of their pyknotic nuclei as well as in the size of the definitely acidophilic cytoplasm, from a diameter equal to that of an erythrocyte to half that and less. Because of the absence of transitional forms between the lymphoid type of cell through the basophil erythroblast and normoblast stages, and because of the variety in size and the absence of a sufficient number of mitotic figures, it seems clear that these were degenerating leukocytes rather than normoblasts. One or two cells were seen that were typical of polychromatic erythroblasts with a heavy "checker-board" chromatin arrangement of the nucleus. However, since the normoblasts in the smear of the blood cultured constituted 0.5 per cent, it is thought that the small number found in the

culture could be attributed only to explanted normoblasts and not to those that had arisen heteroplastically from a lymphoid precursor in the culture.

After sixty hours, migration had continued at a slower rate. The cells in the zone of migration had increased in size, and many of them were fibroblast-like. The nuclei were large and pale, with dustlike particles of chromatin arranged in a fine network; the nuclear membrane was smooth in contrast to the lobulated and wrinkled membrane of the small polyblasts, and one, or occasionally two, large nucleoli were prominently acidophilic. There were many cells similar to these elongated polyblasts, with irregular or plump, oval nuclei and an ameboid cytoplasm, which was pale gray and very fine, and contained reddish-purple granules. These were thought to be ameboid myelocytes. A rare polymorphonuclear cell was present with a somewhat pyknotic nucleus. There were many dead cells with shrunken acidophilic cytoplasm, and pyknotic nuclei were scattered among the polyblasts.

Within the explant were frequent mitotic figures. At the periphery the cells were slightly elongated, while in the center they had maintained their rounded shape. The nuclei were large and vesicular, contained one or two nucleoli, had a fine chromatin arrangement and a heavy, frequently deeply indented nuclear membrane. In many cells the cytoplasm was vacuolated and contained ingested particles. Sparsely distributed through the culture were deeply pyknotic nuclei of dead cells; again, in the areas where there were dense groups of erythrocytes these cells resembled normoblasts. But here, too, the absence of transitional forms from lymphoid cells and basophilic erythroblasts and normoblasts, and the variation in size of the cells, were evidence against erythropoiesis *in vitro*.

After eighty-eight hours, the elongated cells in the zone of migration had developed very long, slender, tapering processes. The cells were not as large or as long as in the sixty hour cultures, and the percentage of rounded and phagocytic cells was higher. Within the explant were many dead cells. Practically all of the living cells were macrophages, many of which contained phagocytosed debris. There were occasional binucleated cells, and many cells had a very large, deeply lobulated nucleus surrounded by a wide margin of vacuolated cytoplasm. No cells with specific granules were seen.

After four days, active phagocytosis was progressing, and as the leukocytes died and were ingested, clear areas remained, into which the polyblasts migrated, becoming then elongated and fibroblast-like. Most of the cells within the explant were very large with vesicular nuclei and vacuolated cytoplasm. Occasional mitotic figures were present.

After six and eight days, the cultures showed large numbers of dead cells, and in contrast with the closely packed cellular explant of the original cultures contained few living cells, most of which were macrophages of great size. The nuclei in these greatly hypertrophied phagocytic cells were relatively small and crowded to one side. Very few spindle-shaped or elongated polyblasts remained. The cultures were heavily speckled with pyknotic nuclei of dying cells. The red blood cells had been ingested or hemolyzed.

In summary, the following findings were significant:

1. On the basis of the study of the dry smears of the blood, the case was diagnosed as myeloblastic leukemia, and the postmortem observations supported this diagnosis.

2. In the sections of the cultures of the buffy coat, it was impossible to differentiate the large majority of cells from lymphocytes.

3. In the young cultures, some of these lymphoid cells hypertrophied and developed granular cytoplasm; they were then classified as myelocytes.

4. Large numbers of the lymphoid cells developed into polyblasts.

5. In the zone of migration of the early cultures, the cytoplasm of many cells, otherwise identical with the ameboid polyblasts, contained granules indistinguishable from specific neutrophilic granules. These cells were interpreted as being ameboid neutrophilic myelocytes. In the older cultures these cells were absent.

6. In the older cultures it was evident that the polyblast had developed into fibroblast-like cells.

7. Within the explant, and among larger numbers of dying leukocytes, the majority of the cells became actively phagocytic and greatly

TABLE 2.—*Blood Counts in Case 3: Acute Myeloblastic Leukemia*

Date	Red Cells	Hemoglobin, per Cent	White Cells	Differential, per Cent						
				Polymorphonuclear Neutrophils	Small Lymphocytes	Medium-Sized Lymphocytes	Transitionals	Myeloblasts	Myelocytes	Eosinophilic Myelocytes
3/10/31	2,840,000	50	104,000	5	15	15	10	6	49	..
3/12/31	2,610,000	45	94,000	3	..	..	..	49	26	2
3/14/31	1,780,000	37	213,000	..	..	..	..	..	..	..
3/16/31	1,530,000	25	149,000	2	14	11	1	29	23	..

hypertrophied. The remaining cells developed large vesicular nuclei and became fibroblast-like.

8. No evidence was found that red cells had developed during the process of culture.

9. The polymorphonuclear cells were actively ameboid and wandered out from the explant, where they began to show evidence of degeneration as early as twelve hours after explantation.

CASE 3.—*Acute Myeloblastic Leukemia*.—R. C., a boy 5 years of age, was admitted to the Evanston Hospital under the care of Dr. H. O. Lussky, on March 10, 1931, in a critical condition. There was a history of pain and swelling in the right elbow one and one-half years previously, with a recurrence after nine months. Seven weeks before entry a croupy cough developed, and a fever of from 100 to 101 F. had been present since that time. Rather severe pain in the lower half of his right leg had developed one week before admission. Physical examination revealed an acutely ill, poorly nourished child, who was extremely pale. The tonsils were very large and edematous, and shotlike generalized lymphadenopathy was present. The spleen and liver were not palpated. The temperature was of septic type. On March 17, 1931, the child died.

The blood cell counts were as shown in table 2.

The clinical diagnosis was myelogenous leukemia.

The anatomic diagnosis as ascertained post mortem by Dr. F. D. Gunn was as follows: high grade hyperplasia of the bone marrow; hypertrophy (moderate) and myeloid metaplasia of the spleen; leukemic infiltration of the liver, kidneys, intestine and myocardium; myeloid metaplasia of the subcutaneous, mediastinal and peripancreatic lymph nodes; multiple small superficial ulcers and macular hemorrhages of the skin; hemorrhage of the gums; hemorrhages of the muscles and fascia of the chest and abdominal wall, endocardium, myocardium, pericardium, peritoneum, pleura, thymus and esophagus; hemorrhages into the lumen of the intestine; mucous polyp of the ileocecal valve; high grade general anemia. Final diagnosis: acute myeloblastic leukemia.

Observations in Leukocyte Cultures: On the day the cultures were made, the blood cell counts were: red, 1,530,000, with hemoglobin, 25 per cent; white, 149,000; differential (on dry smear stained with May-Grünwald-Giemsa), 9.5 per cent myeloblasts, 60 per cent promyelocytes, 7.5 per cent neutrophilic myelocytes, 3.5 per cent metamyelocytes, 1 per cent polymorphonuclear neutrophils, 16 per cent small lymphocytes, 2 per cent erythroblasts and 1 per cent normoblasts. On searching the smear, only 2 platelets were seen.

The promyelocytes were very large, being twice the diameter of an erythrocyte, and had a smooth oval or plumpy lobulated nucleus with a fine chromatin arrangement; the separation of chromatin and parachromatin was not distinct, and a narrow rim of bluish-gray cytoplasm was speckled with a few small, bright red granules. The myelocytes were similar, except that the nucleus was more frequently round, and there was a dense accumulation of neutrophilic granules at one side of the cytoplasm. The myeloblasts could not be sharply distinguished from the promyelocytes, and were similar except for the absence of granules. The metamyelocytes had fine neutrophilic granules as had also the rare polymorphonuclear cell that was present. Rarely a basophilic erythroblast, a very large, deeply basophilic cell, round in outline and having a large nucleus characterized by a heavy chromatin pattern, was seen. Smaller erythroblasts with polychromatic cytoplasm and typical normoblasts were rare. Small mononuclear cells, the size of an erythrocyte, with a heavy chromatin pattern in the nucleus and blue cytoplasm, were lymphocytes.

In the control cultures, fixed immediately after explantation, the majority of the cells were myelocytes. The nucleus in many of them was deeply lobulated; in others, it was smoothly oval, and fine neutrophilic granules could be seen in the cytoplasm. Few polymorphonuclear neutrophils were seen. Many large, round cells with oval or indented nuclei and basophilic cytoplasm were probably those that were called myeloblasts in the dry smear, and those that were smaller with narrow rims of basophilic cytoplasm were indistinguishable in section from small lymphocytes. No normoblasts were seen.

After two hours of incubation, active ameboid migration had occurred. A relatively wide ring of ameboid forms surrounding the explant consisted chiefly of elongated cells with finger-like pseudopodia. The nuclei in these cells presented bizarre shapes; some were elongated; others were dumb-bell-shaped, spindle-shaped, or deeply wrinkled. The cytoplasm was lightly basophilic. These cells were typical of the early stages in the development of polyblasts. A few polymorphonuclear neutrophils were present in this zone. Within the explant, some of the cells had not changed; others were apparently dying, having deeply pyknotic nuclei, and many were definitely hypertrophied.



After five hours, the ameboid cells in the zone of migration were larger, although there was a higher percentage of rounded cells than in the two hour cultures, especially adjacent to the explant. The ameboid cells had either oval or rounded, deeply lobulated nuclei, and abundant pale grayish-blue cytoplasm containing a few almost imperceptible red granules. In the cytoplasm of a few of the elongated, and in most of the rounded, cells were many dull red granules, which varied in size from those at the limit of visibility, to definite, easily distinguishable ones. A few cells with large, deeply indented or fissured nuclei were present; these, too, had a few scattered fine red granules of a variety of sizes in the cytoplasm. There were very few phagocytic cells in the zone of migration, and in each of these the single, crescent-shaped nucleus was crowded to one side by large vacuoles in the cytoplasm, which also contained small granules. Many small cells with pyknotic nuclei were scattered among the ameboid cells.

Within the explant, the living leukocytes were rather sparsely distributed among erythrocytes and cells with deeply pyknotic nuclei. Practically all of these living cells were larger than those in the control, or two hour, cultures. Many phagocytes containing ingested cellular debris and many myelocytes with round or deeply lobulated nuclei were present. A few myeloblasts were still to be seen. Mitotic figures were scarce, and an occasional cell with two small, round nuclei was seen.

After eighteen hours, migration had continued, and the ameboid cells presented a great variety of shapes and sizes. In some fields the majority were greatly elongated, with long, slender, finely granular cytoplasmic processes, and spindle-shaped or dumb-bell-shaped nuclei. In other fields, more of them were rounded ameboid cells with the polyblast type of nucleus and abundant, irregularly outlined cytoplasm. Several polymorphonuclear cells with lobulated and constricted nuclei and acidophilic cytoplasm were seen. A few of the ameboid cells were phagocytic and contained ingested red blood cells. Within the explant, the leukocytes were scattered through the dense collection of red cells. Most of the hypertrophied cells and some of the smaller cells were phagocytes, which contained digested debris. The rest were myelocytes and myeloblasts. A few mitotic figures were seen. No erythropoiesis was found.

After twenty-nine hours, the zone of migration had widened still farther. In addition to the previously described elongated and rounded, polyblast-like cells, there were many large, round myelocytes, each having a large, round or oval, deeply stained nucleus and slightly acidophilic cytoplasm, in which fine neutrophil granules were present. Many of these cells were also phagocytic, containing ingested erythrocytes and nuclear debris. Occasional mitotic figures were present in the zone of migration. A few dying cells, with acidophilic, round cytoplasm and shrunken, broken or round pyknotic nuclei were seen.

Within the explant, many mitotic figures and occasional binucleated cells were seen. Active phagocytosis by mononuclear cells was occurring; most of these cells had granules in their cytoplasm. Many very large, round cells, some of which had nongranular, basophilic cytoplasm, and others of which had slightly acidophilic, definitely granular cytoplasm, were present. Polymorphonuclear cells were rare within the explant. A large number of small cells had pyknotic nuclei.

After fifty-four hours, most of the cells in the zone of migration were rounded, and many cells were dying. A few large myelocytes with large, oval nuclei and granular, acidophilic cytoplasm were still present. Active phagocytosis by spherical cells with relatively small, crescent-shaped nuclei and a large quantity of vacuolated cytoplasm was taking place; granules were inconstantly present in these cells. These phagocytic cells had not reached the size of those at the same stage of cultivation in case 2. A few polymorphonuclear cells were still present.



#### EXPLANATION OF PLATE

All the figures are drawn at the same magnification (Zeiss 2 mm., apochromatic objective,  $20\times$  ocular) with camera lucida at microscope stage level.

Fig. 1.—Two lymphocytes from the peripheral blood just before explantation. Dry smear, stained with May-Grünwald-Giemsa stain.

Fig. 2.—A portion of a section through the buffy coat of the centrifugated blood used for explantation, showing the size and homogeneity of the cells.

Fig. 3.—A section through a twenty-four hour culture, showing an apparently unchanged small lymphocyte, several medium-sized and one very large lymphocyte.

Fig. 4.—A similar section showing three unchanged lymphocytes, two monocyteoid cells and one exceedingly large lymphocyte with very prominent acidophil nucleolus.

Figures 2, 3 and 4 are from preparations fixed in solution of formaldehyde plus Zenker's fluid and stained with hematoxylin-eosin-azure.



In summary of the morphologic changes observed in the cultures of these cells from myeloid leukemia, in which the large majority were myeloblasts and promyelocytes, the following points were significant:

1. In the earliest cultures, the explant was surrounded by a wide zone of ameboid cells, which were morphologically identical with polyblasts.

2. Shortly afterward, many of these actively phagocytic polyblasts contained numerous reddish-purple granules, which varied in size from that at the limit of visibility to that of eosinophilic granules.

3. Some myelocytes contained ingested cellular débris.

4. Since a large proportion of the cells in the early cultures had granular cytoplasm, it was impossible to say definitely that there had been maturation of myeloblasts into myelocytes, although this seemed probable.

5. The majority of the cells in the cultures were actively ameboid and phagocytic.

6. The nature of the granules, which resembled neutrophilic granules, except for their variation in size, is debatable. It is possible that they were similar to the so-called toxic granulation of the neutrophilic leukocytes during infections.

CASE 4.—*Acute Myeloblastic (?) Leukemia*.—W. P., a man 33 years of age, entered the Billings Hospital, March 18, 1931, with the history of a rather insidious onset of headache, oliguria, lumbar pain and constipation ten weeks previously. About three weeks later, he noticed swellings on each side of his neck, which were painless and grew progressively larger. Some drainage had occurred from the one under his ear. Ten years previously, following an attack of measles, he had had swollen glands in the neck, which persisted for about six weeks. Seven years later, three nodules developed in the submental region, which subsided after nonspecific treatment with arsphenamine.

The essential findings on physical examination were those of an acutely ill young adult who was markedly anemic and feverish. There were two draining sinuses on each side of the neck and two just to the right of the midline. The neck was obviously deformed by the subcutaneous swellings. On palpation, several discrete nodules, ranging in size from that of a pea to that of a goose egg, were felt in the chain of lymph nodes posterior to the sternocleidomastoid muscles, as well as many bean-sized nodes in the supraclavicular spaces. The lymph nodes in the axillary, epitrochlear and inguinal regions were palpable also. There was evidence of the presence of fluid in the pleural cavities. The spleen was palpable 8 cm., and the liver 4 cm., below the respective costal margins.

The illness ran a febrile course with peaks of fever of 104 F. for a few days, and then the temperature lowered gradually. The contents of the pleural cavities were aspirated several times for relief of dyspnea, the fluid being sterile each time and showing a cellular content similar to that of the blood. Nodules in the sternum suggesting metastases developed, but this interpretation was not borne out by the roentgen findings. The patient was discharged on May 2, 1931, and died two months later. No autopsy was done.

The blood findings of Dr. Ernestine Kandel, the attending hematologist, are given in table 3.

Dr. Kandel felt that the large number of immature forms seen during the acute febrile stage following admission were myeloblasts, and the diagnosis of acute myeloblastic leukemia was made. As the acute symptoms subsided, the immature forms appeared to be more typical of lymphocytes. She then felt that the condition was chronic lymphatic leukemia with a myeloid reaction during the febrile stage.

Observations in Leukocyte Cultures: On the day of obtaining the blood for culture, March 24, 1931, the cell counts were: red, 1,480,000; hemoglobin, 32 (Dare); white, 250,000; differential (300 cells), 85.3 per cent myeloblasts, 3 per cent metamyelocytes, 3.6 per cent polymorphonuclear neutrophils, 1 per cent promyelocytes, 1 per cent neutrophilic myelocytes, 0.5 per cent eosinophilic myelocytes, 5 per cent small lymphocytes, 0.3 per cent normoblasts and 0.3 per cent mitotic figures (in myeloblasts).

TABLE 3.—Blood Counts in Case 4: *Acute Myeloblastic (?) Leukemia*

Date	Hemoglobin, per Cent	Red Cells	White Cells	Differential, per Cent								
				Neutrophils	Eosinophils	Basophils	Small Lymphocytes	Blasts	Promyelocytes	Neutrophilic Myelocytes	Metamyelocytes	Immature Lympho- cytes
3/18	35	1,500,000	300,000	3	..	..	4	84	3	..	..	6
3/24	32	1,400,000	250,000	3.6	..	..	5	85	1	1.5	3	..
3/30	32	1,480,000	320,000	..	..	..	1.5	76.5	5	4	4.5	8.5
3/31	..	.....	330,000	14	1	..	22	27	..	1	6	19
4/ 6	30	1,500,000	280,000	5	..	..	31	36	1	1	2	24
4/15	30	1,100,000	270,000	20	1	..	54	13	..	1	5	6
4/20	30	1,490,000	300,000	..	..	..	45	37	10	3	..	5
4/28	..	.....	.....	15	..	1	23	21	2	..	4	34
5/ 1	..	.....	.....	10	..	..	64	2	..	..	..	24

The myeloblasts in dry smears were round cells about one and a half times as large as an erythrocyte, each with a round or oval nucleus containing from one to three nucleoli and a fine chromatin pattern. The nuclear membrane was thin but distinct. In most of these cells, the nucleus was slightly eccentric and was surrounded by a halo, wider on one side, in which azure granules were prominent. In a few of these cells, fine, almost imperceptible neutrophilic granules could also be seen in this halo. The cytoplasm beyond the halo was basophilic and frequently finely vacuolar. The neutrophilic myelocytes had a round or slightly oval nucleus; the cytoplasm was filled with very fine neutrophilic granules together with a few scattered, more prominent, azure bodies. The metamyelocytes were characterized by a round or elongated oval nucleus with a heavy chromatin pattern, and the presence of specific neutrophilic or eosinophilic granules in the cytoplasm. The small lymphocytes had a small, oval nucleus with a heavy chromatin pattern and a pale blue rim of cytoplasm, in which were seen a few bright azure granules. The normoblasts seen in the smear were typical, with polychromatic cytoplasm and pyknotic nuclei. Occasional mitotic figures were found among the myeloblasts.

The control sections of the buffy coat, fixed at the time of explantation, consisted of a mass of leukocytes with small clumps of erythrocytes scattered throughout the clot. Although there was a fairly equal distribution of the various cells

throughout the tissue, in some areas cells of one type were seen in groups. This was especially true of the erythrocytes and of the polymorphonuclear cells, while the mononuclears were more evenly distributed throughout the sections. The specimen was made up for the most part of large lymphoid cells, varying in size from that of an erythrocyte to twice that size. The nuclei were large, oval or indented, occasionally horseshoe-shaped, with from one to three large nucleoli, a fine chromatin pattern and a thin, but distinct nuclear membrane. The cytoplasm was basophilic. There were frequent cells in which there was a relatively large amount of slightly acidophilic cytoplasm containing varying numbers of fine neutrophilic granules, and a round or horseshoe-shaped nucleus. Frequent eosinophilic cells, most of which were myelocytes, were prominent because of their bright red granules. Occasional ameboid figures were present. Relatively few polymorphonuclear cells were seen among the massed round cells, although in some areas they were numerous. A few smaller cells in which the nuclei stained deeply and had a heavy chromatin pattern were interpreted as being small lymphocytes. Very few normoblasts with polychromatic cytoplasm and round nuclei were seen.

After seven and a half hours, there was a wide zone of migration, the outer edge of which was made up almost entirely of polymorphonuclear neutrophils. Nearer the explant, rounded myelocytes were scattered among the polymorphonuclear leukocytes. Here, too, were seen elongated cells, each with finger-like processes of finely granular cytoplasm and an oval or triangular nucleus containing from one to three nucleoli at one pole of the cell. Frequent, large, round cells with large, horseshoe-shaped nuclei and basophilic cytoplasm resembled monocytes. Occasional eosinophilic leukocytes with round, indented or lobulated nuclei were present. Within the explant, the changes were less marked, but hypertrophy of the majority of the cells had occurred. Their cytoplasm had increased in amount, and in many the nucleus had assumed a slightly eccentric position. While most of the cells appeared larger than in the control tissue, there were also many apparently unchanged lymphoid cells.

After twelve hours, the zone of migration was wider, and there was more variation in the cell forms. The outer edge of the zone was made up chiefly of polymorphonuclear neutrophils. Nearer the explant the number of rounded and elongated cells with granular cytoplasm and oval or kidney-shaped nuclei had increased greatly. Occasional very long, slender ameboid cells with basophilic cytoplasm in which fine granules were distinguishable were seen at the edge of the explant. Frequent eosinophilic myelocytes were present. An occasional medium-sized hemocytoblast with a large, plump nucleus containing from one to three nucleoli outlined by a distinct nuclear membrane and surrounded by a rather wide border of deeply basophilic cytoplasm lay among the ameboid cells.

Within the explant, frequent eosinophilic myelocytes were recognized among the closely packed leukocytes, among which were many cells with finely granular cytoplasm, identified as neutrophilic myelocytes. Rare mitotic figures were seen, and some of the cells were dead.

After twenty-four hours, many of the cells had died. At the outer edge of the zone of migration the polymorphonuclear neutrophils were still the predominating type, although some of them had pyknotic and broken nuclei. Nearer the explant were large numbers of round cells, the majority of which had vesicular nuclei, containing one or more large oxyphilic nucleoli, and narrow rims of non-granular basophilic cytoplasm. Scattered among them were numerous monocytoid cells with deeply indented nuclei and large amounts of nongranular cytoplasm. There were also many round cells with eccentric nuclei and finely granular cyto-

plasm, which were probably metamyelocytes; some of these had band-shaped nuclei and were ameboid. A few elongated cells with nongranular cytoplasm were also present.

At the edge of and within the explant, the majority of the cells had each a large nucleus with from one to two prominent nucleoli and a distinct, although thin, nuclear membrane surrounded by a narrow rim of nongranular basophilic cytoplasm. However, in many cells of this type, fine reddish-purple granules were seen within the cytoplasm. A few cells of the hemocytoblast type were present at the edge of and within the explant. Frequent eosinophilic myelocytes and metamyelocytes were scattered throughout the culture. A few mitotic figures were seen. An occasional phagocytic mononuclear cell, the cytoplasm of which contained ingested debris and the nucleus of which was crowded to one side, was found within the explant.

After thirty-one hours, the zone of migration was still made up chiefly of polymorphonuclear neutrophils. Near the explant lay more of the rounded and elongated myelocytes than at twenty-four hours. The cells tended to be arranged in clumps, in which the polymorphonuclear leukocyte was the predominating type, together with one or two very large, round cells with vesicular nuclei and basophilic nongranular cytoplasm and a few neutrophilic myelocytes and metamyelocytes. No mitotic figures were seen in these groups of cells. Occasional phagocytes with nongranular cytoplasm containing particles of debris were present among the dying cells at the edge of and within the explant.

Within the explant, the large majority of the cells were round. They were larger than the ones in the original explant, and the number of cells with finely granular cytoplasm was greater. There were many transitional forms between the nongranular, round cells, myeloblasts, finely granular neutrophils, myelocytes, metamyelocytes and polymorphonuclear cells. No mitotic figures were seen in the explant.

After fifty-six hours, in the zone of migration many of the polymorphonuclear cells were dead; a few were living, especially in the area near the explant. In this region were many transitional forms, myelocytes, metamyelocytes, band-shaped polymorphonuclears and their lobulated nuclei. Frequent elongated cells with round nuclei and irregular, granular cytoplasm were seen.

Within the explant, the number of phagocytic cells had increased greatly. These active macrophages were large and nongranular, and contained varying amounts of cellular debris. Within the cytoplasm of some were seen engulfed eosinophils and smaller lymphoid cells, the nuclei of which still maintained their chromatin markings and nucleoli. The majority of the living cells in the cultures were definitely larger than those originally explanted. Many cells of the hemocytoblast type were seen; these were large cells from two to three times the diameter of adjacent erythrocytes, and each had a large, oval or kidney-shaped nucleus with a large clump of chromatin and an abundance of deeply basophilic cytoplasm. They were not as large as the hemocytoblasts found in case 1, however. Although the majority of the cells in the explant were lymphoid in type, there were frequent transitional forms to be seen, from the neutrophilic myelocytes, metamyelocytes and polymorphonuclear cells, and a smaller number of similar eosinophilic cells.

After seventy-two hours, large numbers of the cells had died, but the majority were living. In the zone of migration a few long, spindle-shaped cells with greatly elongated processes were seen, in addition to the ameboid myelocytes and polymorphonuclear neutrophils. Within the explant were many eosinophilic and neutrophilic myelocytes and metamyelocytes. A few mitotic figures were seen in these cells. Many nongranular phagocytes were present. Although there were some cells that had not changed in appearance from the explanted ones, the majority of them had increased in size.

In summary of the findings in this case, in which 85 per cent of the cells in the differential smear were considered to be myeloblasts, it may be said:

1. Some of the myeloblasts seemed to mature into myelocytes and polymorphonuclear leukocytes.

2. Some of the smaller myeloblasts hypertrophied into large ones (hemocytoblasts).

3. Frequent macrophages were seen after twenty-four hours. These contained as many as four erythrocytes.

4. A few long, spindle-shaped or stellate-shaped cells appeared in the fifty-six hour and later cultures.

5. No evidence of erythropoiesis was observed.

The explanted cells behaved, then, in culture like those of myeloid leukemia. The question arises as to whether the cells in the dry smear and the blood in the later course of the disease were myeloblasts rather than lymphoblasts.

CASE 5.—*Chronic Lymphatic Leukemia*.—H. I., a white man, aged 60, entered the Billings Medical Clinics with a complaint of tinnitus. On physical examination, generalized lymphadenopathy and splenomegaly were found. He is still under observation. His general condition is fairly good. The clinical course and findings, as well as the blood picture, are those of chronic lymphatic leukemia.

Observations in Leukocyte Cultures: At the time of removal of the blood for culture, the cell counts were: red, 4,440,000, with hemoglobin 13 per cent; white, 219,000; differential, 1.5 per cent polymorphonuclear leukocytes, 86.5 per cent small lymphocytes and 12.5 per cent medium-sized lymphocytes.

In the control culture, fixed shortly after explantation, the explant was seen to be a dense mass of small lymphocytes, with a typical heavy chromatin pattern in the oval nuclei and basophilic cytoplasm. A few polymorphonuclear cells were sprinkled sparsely through the culture. At the edge of the explant were a few cells each of which had a greater amount of cytoplasm and a slight indentation of the nucleus. No monocytes were seen.

After four hours, there had been little if any change in the appearance of the cultures other than an occasional round small lymphocyte which had migrated from the explant.

After sixteen hours, there were large numbers of small lymphocytes in the zone of migration which had maintained their round shape and also the normal nucleus-cytoplasm relationship. A very few monocytoïd cells with abundant cytoplasm and an occasional medium-sized lymphocyte were present. A few polymorphonuclear neutrophils and eosinophils were seen. Within the explant there had been little change.

After twenty-four hours, the zone of migration contained a large number of cells. In some of the cultures, the cells in this zone were chiefly small lymphocytes and large monocytes with an occasional polyblast; in other cultures, the zone of migration contained large numbers of dumb-bell-shaped and elongated polyblasts with blunt, long pseudopodia and faintly basophilic cytoplasm, in addition to small lymphocytes and rounded polyblasts. A few large cells with small, round or oval



nuclei and finely vacuolated cytoplasm were seen both in the zone of migration and at the edge of the culture. Some of these contained ingested débris. Many of the cells in the cultures were dying and had pyknotic and broken nuclei.

After seventy-two hours, most of the cells were dying. In the zone of migration there were many ameboid forms, and some of the cells were greatly elongated. Many small and a few large macrophages were present. There were many unchanged cells still present in the explant.

In summary, the changes observed in the cultures of the cells of this case of chronic lymphatic leukemia were:

1. Many of the lymphocytes disintegrated very early, although most of them remained unchanged.
2. Some lymphocytes migrated and developed into rounded and elongated polyblasts, which later became phagocytic.
3. A few large monocytes were seen.
4. No mitotic figures were seen in the cultures.
5. No hemocytoblasts were found.

CASE 6.—*Chronic Myelogenous Leukemia*.—S. D., a Negro, aged 18, was admitted to the Cook County Hospital on Nov. 12, 1930, with a history of insidious onset of loss of weight, dyspnea, enlargement of the abdomen, recent epistaxis, difficulty in hearing and pain in the back. On entrance examination there were evidences of fluid in the chest, palpable lymph nodes, an enlarged spleen, and hemorrhages from the mucous surfaces. The red count was 2,300,000; the total white count, 453,000, with a large number of myelocytes and polymorphonuclear cells. A typical leukemic retinitis developed. The patient suffered repeated hemorrhages from the mucous surfaces. Against advice, on March 24, 1931, he was taken from the hospital.

Observations in Leukocyte Cultures: At the time of obtaining the blood for cultures, the cell counts were: red, 2,300,000, with hemoglobin 50 per cent; white, 453,000; differential, 4 per cent band-shaped and 27 per cent segmented polymorphonuclear leukocytes, 4.5 per cent myeloblasts, 52 per cent neutrophilic myelocytes, 3 per cent eosinophilic metamyelocytes, 3 per cent myelocytes, 2 per cent micro-myelocytes, 3.5 per cent normoblasts, 1 per cent basophilic erythroblasts, 0.5 per cent basophil leukocytes and no lymphocytes.

In the dry smear, the neutrophilic myelocytes were large cells, ranging from one and one-half to two and one-half times the size of an erythrocyte. The nuclei were large and had an almost imperceptible nuclear membrane and a fine chromatin network, and in the more deeply stained cells, from one to two nucleoli. The cytoplasm was filled with very fine neutrophilic granules. In most of these cells azure bodies were also present. The myeloblasts were similar large cells with faintly basophilic, reticular cytoplasm without neutrophilic granules, although azure granules were constantly present. The eosinophilic myelocytes and metamyelocytes had the characteristic large, red granules packed closely in the cytoplasm. The micromyelocytes were the size of an erythrocyte, had an oval nucleus, fine neutrophilic granules in the cytoplasm, and large, azure bodies sprinkled throughout the cytoplasm. The normoblasts had a heavy chromatin arrangement in the small, round nucleus, and an irregular, smooth, reddish-yellow cytoplasm. The basophilic erythroblasts were about one to one-half times as large as an erythrocyte and had a deeply basophilic cytoplasm and a large nucleus, which had a fine chromatin arrangement and usually one nucleolus. The basophilic leukocytes had the characteristic large, bluish-purple granules in the cytoplasm.

In the control sections of the buffy coat, the large majority of the cells were only slightly larger than an erythrocyte, being rounded and not flattened as in the smear. The nuclei were round or oval, had each a definite nuclear membrane, fine chromatin arrangement and from one to three nucleoli. The cytoplasm in most of the cells was acidophilic and vacuolar, and fine neutrophilic granules were seen with difficulty. A few cells were larger, but otherwise similar. Many eosinophilic myelocytes were prominent because of their large, red granules. Many polymorphonuclear cells were scattered among the round cells.

After six hours, the explant was surrounded by a wide zone of migration, made up of neutrophilic, and fewer eosinophilic, polymorphonuclear leukocytes. The migrating cells had reached the edge of the clot and were crowded closely together there. Within the explant most of the cells were larger than those in the control explant. Most of them were neutrophilic myelocytes, among which a few mitotic figures were seen. A few polymorphonuclear neutrophils were present. A few normoblasts with irregularly polychromatic cytoplasm and shrunken, round, pyknotic nuclei were easily recognized among the leukocytes.

After twenty-one hours, the zone of migration was still composed chiefly of polymorphonuclear neutrophils and eosinophils, especially at the periphery of the zone. Nearer the explant were many large myelocytes, the cytoplasm of which was often vacuolated, and the nuclei of which were large and round or oval, contained from one to three nucleoli, and had a fine chromatin pattern with a distinct nuclear membrane. More of the elongated myelocytes were present than in the six hour cultures. Frequent metamyelocytes with band-shaped or round nuclei and granular cytoplasm were seen scattered among these round cells. Within the explant there had been hypertrophy of many of the cells. The cytoplasm had increased in amount and was finely granular, and many of the cells had plump horseshoe-shaped or deeply bilaterally indented nuclei. Fewer mitotic figures and few normoblasts were seen here than in the earlier cultures.

After thirty and forty-five hours, the number of myelocytes and metamyelocytes in the zone of migration had increased further. It was possible to find all intermediate forms between the smaller myelocytes in the explant and the larger myelocytes and metamyelocytes in the migration zone. Within the explant, the majority of the cells were myelocytes. A few mitotic figures were present. More metamyelocytes and polymorphonuclear cells were present than in the twenty-one or thirty hour cultures. A few normoblasts could be distinguished from the dying myelocytes by their irregularly shaped, polychromatic cytoplasm and round, pyknotic nucleus. An occasional cell contained small particles of phagocytosed debris. After three days, the cultures showed evidence of death of many of the cells. In the zone of migration, polymorphonuclear cells and round metamyelocytes were in great predominance, while few ameboid myelocytes were seen. Within the explant, the majority of the cells were myelocytes, but large numbers of metamyelocytes and many polymorphonuclear cells were present. No normoblasts were identified; many dying cells were seen. After four days, the cultures contained few living cells, most of which were metamyelocytes and polymorphonuclear leukocytes. A very rare round macrophage was seen. Shadows of cells and cells with pyknotic nuclei were frequent.

In summary, the following observations were made:

1. Evidence was found that myelocytes mature in culture into metamyelocytes and polymorphonuclear cells.
2. No evidence of erythropoiesis was present.

3. Very few phagocytic cells developed in these cultures, which consisted for the most part of myelocytes and polymorphonuclear cells.

CASE 7.—*Chronic Myelogenous Leukemia*.—J. B., a man 50 years of age, entered Michael Reese Hospital on Nov. 12, 1931, under the care of Dr. H. Bunswanger, with a complaint of pleurisy pain of prolonged duration and poor vision. Six months previously, while he was under observation for the eye trouble, a leukemic blood picture was found, the total white cell count being, then, 165,000 with a large number of myelocytes. At the time of entry in November, he was not acutely ill and no lymphadenopathy was present, but the spleen was markedly enlarged. The blood cell counts on Nov. 17, 1930, were: white, 510,000; differential, 10 per cent polymorphonuclear neutrophils, 24 per cent immature leukocytes (band forms), 38 per cent promyelocytes, 9 per cent myeloblasts, 29 per cent myelocytes and 2 per cent normoblasts. He was discharged from the hospital on Nov. 26, 1931, in good condition, with orders to return for roentgen treatment at a later date.

Observations in Leukocyte Cultures: The blood cell counts on the day of culture were: red, 3,900,000, with hemoglobin 70 per cent; white, 165,000; differential, 10 (2.2 per cent) large myeloblasts, 178 (44.5 per cent) small myeloblasts, 64 (16 per cent) small neutrophilic myelocytes, 5 (1.2 per cent) large neutrophilic myelocytes, 19 (4.7 per cent) metamyelocytes, 39 (9.9 per cent) band-shaped, 79 (19.7 per cent) mature polymorphonuclear neutrophils, 3 (0.7 per cent) eosinophilic myelocytes and 3 (0.7 per cent) small lymphocytes.

In the dry smears, the myeloblasts resembled large and medium-sized lymphocytes; the nucleus was difficult to demarcate from the somewhat vacuolar basophilic cytoplasm, had a fine chromatin pattern, and from two to three vague nucleoli. No granules were seen in the cytoplasm. The small myelocyte had a large, bean-shaped nucleus with a more definite nuclear membrane and one to two nucleoli; the cytoplasm was slightly acidophilic, and fine neutrophilic granules were present on the side of the indentation of the nucleus. In the larger myelocyte, the nucleoli were more prominent, and scattered throughout the cytoplasm were frequent azure bodies in addition to the fine neutrophilic granules.

The control sections of the buffy coat consisted for the most part of mononuclear cells, the greatest number of which were about one and a half times the size of an erythrocyte; each had basophilic cytoplasm and a large nucleus with a fine chromatin network, a distinct nuclear membrane and from one to three nucleoli. These were the cells that were called myeloblasts in the differential dry smear. There were also large numbers of cells with similar nuclei, frequently eccentrically placed, in the cytoplasm of which fine neutrophilic granules were visible, and these were recognized as neutrophilic myelocytes. A few eosinophilic myelocytes and metamyelocytes were present. Frequent neutrophilic metamyelocytes and band-shaped and mature polymorphonuclear cells were scattered throughout the tissue. A rare cell, with a round pyknotic nucleus and an irregular polychromatic cytoplasm, was identified as an erythroblast. There were a few large, round cells, each with a nucleus containing two or three nucleoli and a heavy nuclear membrane, in which no granules were seen in the slightly basophilic cytoplasm.

After two hours' incubation, the culture was surrounded by a zone of migrating cells, which was made up for the most part of polymorphonuclear neutrophils. Near the explant were numerous neutrophilic myelocytes. Within the explant there had been little change in the cells since explantation.

After ten hours, the zone of migration had increased greatly in diameter. While the majority of the cells were ameboid polymorphonuclear neutrophils, there were also seen similar cells in which the nucleus was not lobulated, but round, wrinkled or oval. Near the explant were many round myelocytes which had wandered from

the explant. Within the explant, the majority of the cells were recognized as myelocytes, having a round or oval nucleus containing from one to three nucleoli, fine clumps of chromatin, and a distinct nuclear membrane surrounded by a finely granular cytoplasm. A few large cells with similar nuclei and nongranular cytoplasm were seen. These resembled large lymphocytes, but were probably myeloblasts. Several metamyelocytes and eosinophils were scattered throughout the explant. No erythroblasts were recognized.

After twenty-four hours, the cells at the periphery of the zone of migration were polymorphonuclear leukocytes and myelocytes. Near the explant were many metamyelocytes, large myelocytes, smaller myelocytes and a few round cells with nongranular cytoplasm. Within the explant, the majority of the cells were myelocytes. Many of these were large and had a relatively greater amount of cytoplasm than those in the earlier cultures. There were a few hemocytoblasts with a large, oval nucleus, containing two or three nucleoli, and basophilic cytoplasm.

In several places in the culture were groups of normoblasts with round, pyknotic nuclei and irregular orthochromatic cytoplasm. A few dying leukocytes were seen, which closely resembled these normoblasts. However, these cells had a round outline and definite acidophilic cytoplasm; their nuclei were of various sizes and shapes, and nuclear debris was present within the cytoplasm. The similarity of dying cells to normoblasts makes it necessary to be guarded in the identification of a cell as a normoblast in blood cultures. Only when the various stages in erythropoiesis could be demonstrated was it considered prudent to identify the cells as such.

After twenty-six hours, the largest number of cells in the zone of migration were ameboid neutrophilic myelocytes and polymorphonuclear neutrophils. There were groups of cells, more frequent at the periphery of this zone, in which there were many transitional forms between the ameboid myelocytes and the polymorphonuclear neutrophils. Within the explant, the majority of the cells were granular; a few were polymorphonuclear, and a few had the horseshoe-shaped nucleus of a metamyelocyte. There were a few mitotic figures in large, round cells in the cytoplasm in which were dull red granules larger than the granules in the majority of the neutrophilic myelocytes and resembling those described in case 3. These granules were also seen in a few of the smaller round cells. A few dying leukocytes, but no normoblasts, were seen in these cultures.

After forty-eight hours, the type of cells in the zone of migration had not changed, but here the transition from myelocytes to polymorphonuclear cells was clearly demonstrated by the presence of a zone of myelocytes immediately surrounding the explant, which merged into a zone where most of the cells were myelocytes, and into a peripheral zone where the largest number of polymorphonuclear cells were seen. No mitotic figures were seen in the zone of migration. Within the explant, the majority of the cells were round myelocytes. A few eosinophilic myelocytes were prominent because of their large, red granules. A few mitotic figures and macrophages were seen.

After three days, many of the cells within the explant and also in the zone of migration were disintegrating. In the polymorphonuclear cells and in the elongated, mononuclear, granular cells, the granules were larger than those in the previous culture and were identical with those described in case 3. Within the explant most of the living cells were large myelocytes containing the same dull red granules, and there were frequent metamyelocytes and a few polymorphonuclears scattered throughout the explant.

After five days, most of the cells in the culture had disintegrated. In the zone of migration, most of the cells were rounded, had irregularly shaped, granular

cytoplasm and dark nuclei. Within the explant, most of the cells had pyknotic, shrunken nuclei and acidophilic cytoplasm, in which granules were seen with difficulty. There were many phagocytic cells having a small crescent-shaped nucleus crowded to one side by a large amount of vacuolated cytoplasm containing ingested debris.

In summary, in cultures of the blood in this case of chronic myelogenous leukemia, the following observations were made:

1. The myeloblasts developed into myelocytes, and the latter matured into polymorphonuclear leukocytes.
2. Doubtful evidence was found which indicated that erythrocytes had developed from a large basophil progenitor.
3. Myeloblasts developed into macrophages in the older cultures.

In four cases of aleukemic lymphatic leukemia in children, one of whom had a total white cell count of 14,000 and the other three of less than 5,000, the cultures of the leukocytes proved unsatisfactory. The cells did not grow well. The lymphocytes disintegrated early, and although in all of the cases a few phagocytes were obtained in the cultures, after thirty-six or forty-eight hours their origin could not be traced.

#### COMMENT AND SUMMARY

The outstanding morphologic changes in the leukocytes in culture were as follows:

1. Pronounced ameboid activity of the cells was observed in all cases of lymphatic and myeloid leukemia.

2. Hypertrophy occurred in the great majority of the explanted cells of all the types of leukemia, except chronic lymphatic and aleukemic leukemia, a large number of the cells of which remained unchanged and showed evidence of early degeneration.

3. Monocytoid cells developed in all cases, the fewest being present in the chronic forms of myeloid and lymphatic leukemia, and the greatest number, in the acute forms of both types.

4. Polyblasts or macrophages were found in all cases, the largest number developing in acute myeloblastic leukemia. In the cases in which these cells were found in largest numbers, fibroblast-like cells also were seen in the later stages.

5. Hemocytoblasts were seen to develop in the acute and chronic myeloid leukemias and also in the acute lymphatic leukemia, in which 96 per cent of the cells were small and medium-sized lymphocytes.

6. Evidence of the maturation of myeloblasts into myelocytes was found in the cases of myeloblastic leukemia.

7. Evidence that myelocytes matured into polymorphonuclear leukocytes in cultures was not conclusive.

8. Evidence of the development of members of the red cell series from a basophilic progenitor (hemocytoblast or myeloblast) was incomplete.

9. Granules similar to neutrophilic granules except for their variation in size and shape were seen to develop in two cases of myeloblastic and one case of chronic myeloid leukemia. The nature of these granules was not determined, but they did not develop in the cases of acute, chronic or aleukemic lymphatic leukemia. They differed from the granules of the X cells of Rhoads and Parker in that they were larger and more irregular in size.

In answer to the questions listed in the introduction of this article, it may be said:

1. Leukemic blood cells in culture undergo many of the same changes that normal leukocytes do in similar preparations.

2. In these experiments, the lymphoblast of acute lymphatic leukemia differed in its behavior in culture from the myeloblast of acute myeloid leukemia by not forming myelocytes. Both of these cells produced monocyte-like cells, polyblasts or macrophages, fibroblast-like cells, and hemocytoblasts.

3. The lymphocytes of chronic lymphatic and aleukemic leukemia did not show the active growth and morphologic changes that were observed in those of acute lymphatic leukemia. Those few cells that did hypertrophy, however, behaved like those of the acute condition.

4. Strong evidence that the small lymphocyte is a cell of various potencies was found. The evidence of previous investigators (Timofejewski and Benewolenskaia, 1926; Maximow, 1928; Caffier, 1927; Bloom, 1928) that this cell has the capacity of ameboid activity and of development into monocyte-like cells, polyblasts and fibroblast-like cells is corroborated. The observation in the present investigation that the small lymphocytes produce large lymphocytes (typical hemocytoblasts) in culture is of great interest. This is confirmatory evidence for the contention of Weidenreich (1911), Dominici (1901-1920), Maximow (1907-1927) and Jolly (1923), who considered the small lymphocyte of the blood to be able to develop under certain conditions into the large lymphocyte, a view that is denied by practically all other hematologists, particularly by Nägeli, Schilling and Ferrata, who considered the small lymphocyte to be completely differentiated. According to the unitarian theory of hematopoiesis as advocated by Maximow, Jolly, Weidenreich, Downey, Jordan and others, the lymphocyte has still other developmental potencies, namely, into (1) the macrophage direction and (2) the erythrocytic and myelocytic series of blood cells. But the latter has not been demonstrated for blood lymphocytes in culture, although the lymphocytes of lymphatic tissue explanted with bone marrow extract developed into myelocytes (Maximow, 1923).

5. The observations of Timofejewsky and Benewolenskaja (1926) that the myeloblasts produced granulocytes, monocytes and fibroblast-like cells were corroborated. However, conclusive evidence of erythropoiesis in the cultures was not observed. It is, of course, possible that the absence of this finding may have been due to unfavorable conditions in my cultures.

6. The finding of cells morphologically identical with monocytes as transitional stages in the development of polyblasts in the cultures of leukocytes of all the types of leukemia is contradictory to the theories that the monocyte arises from a specific stem cell. The present understanding of the origin of the monocyte is admittedly one of great confusion, owing to the fact that various theories have been built up without sufficient observations of the origin of the cells *in situ* (Bloom, 1928). The main theories now actively debated fall into four main groups: 1. Some investigators (Nägeli, 1923) believe that the monocyte originates in the bone marrow from the myeloblast. 2. Others (Cunningham, Sabin and Doan, 1925) assert that the monoblast, normally present in the spleen and connective tissues, is the specific forerunner of the monocyte. 3. Others (Weidenreich, 1911; Maximow, 1927; Bloom, 1928) feel that the monocyte is a modified lymphocyte or hemocytoblast. 4. Others (Aschoff, 1913; Schilling, 1929) contend that the monocyte is a derivative of the histiocyte. 5. Still others (Downey, 1931) ascribe to it an origin from several of these sources. The findings in this investigation substantiate the belief of other investigators using the tissue culture method, namely, that the monocyte can develop into a macrophage, and support the observation of Maximow (1925-1928), Bloom (1928), Caffier (1927), and Timofejewsky and Benewolenskaja (1926) that in the development of a lymphocyte into a macrophage the lymphocyte passes through a monocyte-like stage. This lymphoid precursor is the much discussed cell called by some the lymphoblast and by others the myeloblast, neither of which can be differentiated from the other in sections or dry smears. This investigation strengthens, then, the contention of the unitarian school that the monocyte is a modified lymphocyte. Until it can be more satisfactorily demonstrated that the lymphocyte can be morphologically differentiated from the monoblast by any of the known histologic methods, including supravital stain, and since the "rosette formation" is not specific for the monocyte (Hall, 1930), it appears that the weight of evidence is in favor of the "monoblast" being a lymphocyte or a myeloblast, or both (i. e., a hemocytoblast).

7. As to whether the tissue culture method may be relied on as a diagnostic aid in the determination of the types of leukemia encountered clinically, it may be said that it is of value in those cases in which the majority of the leukocytes of the blood are of the large, undifferentiated type. When it is impossible in dry smears to differentiate the

"myeloid cell" or a "lymphoid cell," and when on growth of leukocytic cultures granular cells are observed to develop, one may conclude that the explanted leukocytes had the potencies commonly recognized as characteristic of myeloid cells. If no granular cells are seen to develop, one may conclude that the cells are of the lymphocytic type.

8. It is not necessary here to review in detail the many theories of the formation of blood, since recent surveys by Jolly (1923), Downey (1928), Maximow (1927), Sabin (1928), Doan (1931) and Bloom (1932) have covered this subject so completely. Suffice it to say that the unitarian view supported by Dominici, Dantschakoff, Weidenreich, Jolly, Maximow, Jordan, Downey, Bloom and others, namely, that the hemocytoblast or large lymphocyte of the blood and lymphatic tissue is endowed with manifold potencies of development, is the theory which gains the most support from this investigation.

The polyphyletic theories supported by Ehrlich, Schilling, Ferrata, Sabin, Cunningham and Doan, Aschoff, Schridde and Nägeli are incompatible with the findings in these cultures. The concept of Sabin, Cunningham and Doan (1925) that vascular endothelium produces the red cell series and the clasmatoocyte, while the primitive reticular cell produces the three stem cells, the lymphoblast, the myeloblast and the monoblast, gains no support except of a negative nature, notably the absence of erythropoiesis in my cultures; but Timofejewski and Benewolenskaja (1926) reported this finding. That the lymphoblast was indistinguishable from the myeloblast, and that monocytes, polyblasts and fibroblast-like cells were derived from both, is evidence against the irreversible differentiation of these blast forms.

This investigation supports the concept that a sharp distinction cannot always be drawn between myeloid and lymphatic tissue (Maximow and Bloom, 1930). What determines the types of cellular response of the blood-forming organs in the various clinical types of leukemia and allied conditions, is a matter at present under active inquiry by numerous investigators.

#### CONCLUSIONS

Detailed accounts are given of the observations made during growth of cultures of leukocytes taken from two patients with chronic myelogenous, one with chronic lymphatic, three with myeloblastic, and one with acute lymphatic, leukemia, the case histories of which are briefly sketched.

In the myeloid and myeloblastic types, myelocytes, polyblasts, fibroblast-like cells, monocytes and hemocytoblasts developed.

In the chronic lymphatic type, a few monocytes, many polyblasts and fibroblast-like cells appeared, although many of the explanted cells remained unchanged.



In the acute lymphatic type, monocytes, polyblasts, fibroblast-like cells and epithelioid cells developed in large numbers, as well as hemocytoblasts, morphologically identical with those found in lymph nodes and bone marrow.

The development of these hemocytoblasts from known small lymphocytes is of hematologic significance, since, according to the unitarian school, in both the embryo and the adult normal body, the free stem cell is a polyvalent cell capable of producing all other types of blood cells.

It is felt that the tissue culture method may prove to be of diagnostic aid in leukemias in which "blast" forms dominate the blood picture.

Dr. William Bloom of the Department of Anatomy of the University of Chicago, in whose laboratory the work was done, gave me his advice and counsel; Miss Ruth Holton, technical assistance.

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# ORIGIN OF TEETH IN DERMOID CYSTS

## SOME REFLECTIONS ON THE ENIGMA OF THE TERATOMA

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Teratoma, with its curious aggregations of tissues closely resembling those of the developing or adult body, has been a source of wonder to all investigators. The extraordinary degree of differentiation of the tissues with formation of organ-like structures has so enthralled the imagination of observers that they seem to have been unable to attack the problem of these bizarre growths except by way of the view that such collections of tissues actually are malformed organs, or attempts at their formation. The writings on teratoma show a wealth of hypothesis, the plausibility of which has led to superficially apt conclusions. In the middle ages, the opinion prevalent was that the growths were malformed fetuses—a judgment of the Deity on immoral practices—and, as happens when a hypothesis comes to be accepted widely, the suggestion was regarded as fact, and immorality was deduced from the presence of pelvic teratoma. The acceptance of this view was unquestioned until it was strenuously opposed by Blumenthal. The death knell was sounded by Baillie<sup>1</sup> in 1789 when he reported an example of dermoid cyst containing teeth and hair, etc., occurring in the ovary of a virgin girl, aged 11.

Until the end of the last century only sporadic reports and observations were made. Then the subject was attacked not only by gross observation but also by histologic examination and experimental investigation. The same point of view—that the growths must be related in some way to a fetus—manifested itself and controlled investigations.

The cystic tumors, particularly those in the ovary, were thought to be obvious malformed embryos. The protuberance of Rokitansky was said to represent the embryo, and the cyst was regarded either as an amniotic cavity or as a dilated graafian follicle. Gross structures were interpreted as limbs, brain, etc., without adequate investigation of their

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1. Baillie, M.: London M. J. **10**:322, 1789.

intimate nature. Histologic examinations resulted in the discovery of a great many tissues grouped together in a more or less orderly manner, and extraordinary conclusions were again drawn, all to fit the hypothesis discussed in a foregoing paragraph. Eyes have been described, as well as brain, trachea, alimentary canal and even such components as an appendix or cecum (Pommer<sup>2</sup>). Examination of structures similar to those referred to or investigation of the original reports and illustrations (when present) causes one to pause in admiration of the fertility of imagination of some of these writers.

Experimental work showed that parthenogenesis of vertebrate ova could be induced in some animals. It was also shown that structures similar to those found in teratoma could be produced by the injection into animals of embryonic tissue. This was all taken as direct evidence, and the case for the heterochthonous development of teratoma seemed to be complete.

The almost invariable presence of nervous tissue and the predominance of structures of ectodermal origin were explained by the hypothesis that intracystic tension prevented the growth of the "fetus" after a time, and that thus those structures which are to be found best developed in the early embryo, namely, ectoderm of the anterior end of the embryo and particularly nervous tissue, are to be found best developed in the tumors. Many difficulties of interpretation arose, since in some cases the structures did not appear to belong to the anterior end of the embryo.

Two pathologists accepted their own observations in preference to the hypothesis and interpreted structures as hind limbs and labia (Shattock<sup>3</sup>) and scrotum (Ingier<sup>4</sup>). This preference is one that needs cultivation particularly when dealing with the teratoma. If one goes so far as to assume, with Shattock, that in some cases the teratoma does not develop in the same way as an embryo—that is, the cell growth is controlled in some way that is quite different from that in which the cell differentiation in the fetus is controlled—it is not difficult to take the further step of realizing that the teratoma possibly does not correspond to a malformed fetus, and that the resemblances are of gross morphologic character and accidental.

Examination of many of the structures that have been "recognized" in these growths shows that purely superficial resemblances are taken as criteria of identity. There is one structure, however, about which there is a great deal of difficulty, namely, a tooth. In all probability, teeth in "dermoids" have been greatly responsible for the development

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2. Pommer, G.: *Centralbl. f. allg. Path. u. path. Anat.* **1**:260, 1890.

3. Shattock, S. G.: *Tr. Path. Soc., London* **58**:267, 1907.

4. Ingier, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **43**:356, 1908.

of the current hypothesis. From the presence of teeth, bones have been identified as maxillae and a cavity as a mouth or, when pseudo-stratified ciliated epithelium was present, as a pharynx showing a junction of alimentary and respiratory tracts. A large superstructure of hypothesis has developed around the tooth.

The presence of teeth appears to support the hypothesis of a malformed parthenogenetic fetus more strongly than other observations. Since so many difficulties are found in the application of the hypothesis to other structures, it is desirable to examine the teeth afresh. Are they actually teeth comparable in every respect with oral teeth?

To begin with, however: What is a normal tooth?

A working definition is as follows: Teeth are highly specialized structures of definite morphology and chemical nature, which are organs of mastication and, to some extent, speech, and which develop by an epithelial downgrowth, at the site of the dental groove, from the squamous epithelium of the oral mucosa.

An examination of "dermoid" teeth shows that the first criterion applies accurately; the teeth correspond to those of the mouth in every way; they present enamel, dentin, cement and a pulp chamber. It is true that the enamel may be laid down in irregular masses, and that the pulp chamber may be unusually large and contain débris in the form of pulp stones, but nevertheless the general features of oral teeth are so faithfully reproduced that one is able to distinguish incisor, bicuspid and molar teeth. The enamel and dentin appear to be identical in structure and nature with those of oral teeth (fig. 6).

When we come to the next criterion, however, there is considerable difficulty. A teratoma is not a separate individual; it obtains its nutriment from the blood vessels of the host without the interposition of a placenta, and there is no possibility of the growth having a separate existence. The development of any tissue in such a growth cannot have purposive significance, even if we were disposed to accept such a hypothesis for development in general. Thus the idea of mastication in a teratoma is almost as great an absurdity as some other suggestions met with in the literature, when taken to their logical conclusions. Why should some growths require three hundred teeth, and why should a tooth occur without any other tissue? Explanations of these phenomena might be found if the teeth were arranged in a "mouth," but they occur in many places, even on the "scalp" of the protuberance. We have seen a single tooth in a small cavity lined by squamous epithelium occurring in a comparatively large growth consisting otherwise entirely of thyroid tissue. It seems futile to say that all of the embryo failed to develop except one tooth and a thyroid gland, which became hyperplastic. It becomes apparent that to main-

tain the hypothesis that the teratoma is an extraneous growth arising from an ovum or from a blastomere, we must superimpose hypothesis on hypothesis.

Thus the conclusion is forced on us that, from this point of view, teeth in a teratoma, though almost identical in structure with those in the mouth, have a different biologic significance; that is, they are not a portion of the mouth of a malformed embryo, but have arisen in tissues of the host by a process of differentiation usually observed only in the region of the mouth.

It is necessary now to examine the mode of development as shown by the relationships of the teeth to surrounding tissues.

Fully formed teeth give little indication of their mode of origin. Their microscopic structure closely resembles that of normal teeth, and they project usually from a surface lined by squamous epithelium and are frequently embedded in bone, though at times only in connective tissue.

They appear thus to fulfil the third criterion of the definition in that they appear to arise from squamous epithelium. It might be argued that the presence of a mouth is merely a question of interpretation, and that, in explanation of teeth on the surface of the protuberance, the mouth of the embryo might be considered to be opened out widely.

Examination of developing teeth in a teratoma completely negatives this view and supports the contention submitted concerning the essential biologic difference of teratomatous teeth from oral teeth.

#### MATERIAL

One typical specimen of a teratomatous ovarian cyst is taken as illustrating the features of these developing organs.

The cyst was  $3\frac{1}{2}$  inches (8.9 cm.) in diameter, contained greasy fluid and had a projection into its cavity from one wall, measuring 2 by 2 by  $1\frac{1}{2}$  inches (5 by 5 by 3.7 cm.). This projection contained a large number of small cysts. These cysts were variously lined by different types of lining, and between them there was tissue containing muscle, bone, cartilage and areolar connective tissue.

In that portion of the protuberance near its junction with the wall of the main cyst there were some small masses of bone and a few small teeth. These were in relationship to some of the subsidiary cysts. One such typical cyst will be described.

This cyst, irregularly oval, lay close both to ovarian stroma at the basal attachment of the protuberance and to the surface of the protuberance, being separated from the cavity of the main cyst surrounding the protuberance, in one part, by tissue only one thirty-second of an inch (0.08 cm.) in thickness.

The tooth related to this cyst and the associated bone lay on one aspect of its cavity, and the tooth was entirely embedded in soft areolar tissue. After the cyst was opened, a small depression in the cyst wall near the tooth, could be seen macroscopically. The wall was lined by a smooth, shining tissue and appeared to

be uniform throughout its extent. The tissues concerned were removed, decalcified and examined by serial sections.

Microscopically, the wall showed various appearances. In greater part, the tissue nearest to the lumen of this cyst consisted of material of a structure identical with tissue of the central nervous system. It is proposed to call it neuroglial tissue, but it is to be appreciated that there is no proof of its nature; it is merely morphologically identical with neuroglial tissue (fig. 2). In some parts, this tissue lined the wall of the cyst. In other parts, on the internal aspect of this neuroglial tissue, there was a pseudostratified ciliated epithelium, which in some places became a single layer of columnar cells (fig. 3). The cells had basally situated nuclei, but at times this polarity was reversed, and a clear area of cell projected toward the subepithelial tissue. There was a fibrillar connection between the



Fig. 1.—Drawing showing the relationship of the developing tooth to the neighboring cyst. The cyst is lined for the most part by nervous tissue (fig. 2), but in part there is an epithelial lining (fig. 3), and in the crypt a portion of this has become squamous. A neighboring cyst also lined by nervous tissue is shown on the left. Ovarian stroma is present above.

epithelium and the subjacent tissue, thus increasing the resemblance to nervous tissue with an internal layer of embryonic epithelium.

In the area of the wall near the tooth there was a definite crypt resembling the dental furrow, and from this down toward the tooth could be seen discontinuous strands of connecting epithelial tissue. Beneath the tooth was a small mass of bone.

More thorough examination revealed a number of astonishing (if one still clings to preconceived notions) accompaniments. Beneath the neuroglial tissue, in addition to the bone, there were fat and other mesenchymal derivatives. In some areas where the neuroglial tissue was thin, the pseudostratified epithelium grew down and formed glands of the mucous and serous types (fig. 4). The resemblance to respiratory epithelium in this part was remarkable. In the depths of the crypt, for a short distance the epithelium became squamous (fig. 5). From this epithelium in the



Fig. 2.—Photomicrograph of a portion of the wall of the cyst showing tissue that morphologically is indistinguishable from nervous tissue;  $\times 90$ .

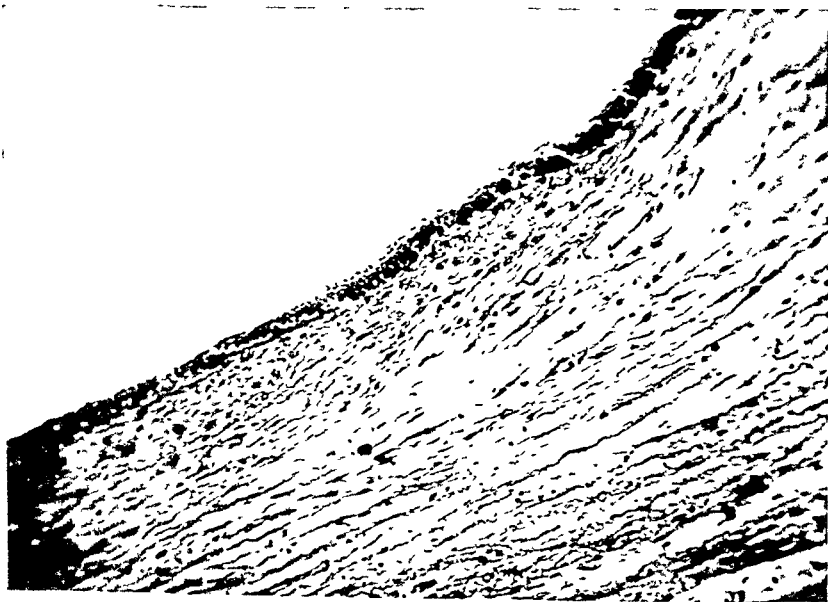


Fig. 3.—Another portion of the cyst, in which ciliated epithelium overlies the nervous tissue;  $\times 90$ .

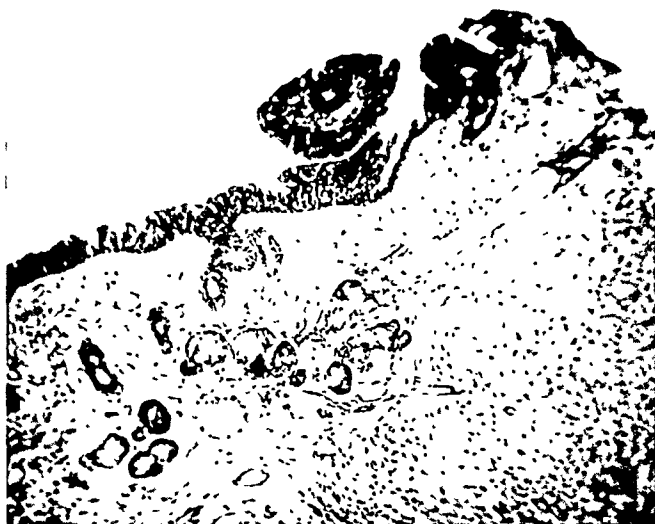


Fig. 4.—Another portion continuous with that shown in figure 3, in which glands have developed;  $\times 40$ .



Fig. 5.—The epithelium of the crypt—on one side squamous;  $\times 90$ .



crypt to the region of the "root" of the tooth there were in the tissue, collections of epithelial cells in strands and groups that imitated closely the epithelial débris of Malassez.<sup>5</sup> The tooth was well formed, except that the amount of enamel was disproportionately large. Parts of its structure are shown in figure 6.

Similar observations were made in the case of other teeth, though minor variations occurred. Some were found to be connected with the surface epithelium, which was of the same type as that seen in the cyst. In a few cases, the amount of squamous epithelium was much greater and showed the development, in addition to the tooth, of subjacent sebaceous glands and hair follicles.

#### COMMENT

In the interpretation of these observations, certain difficulties that are inherent in all cases of both macroscopic and histologic examination arise.



Fig. 6.—Portion of tooth showing ameloblasts and enamel;  $\times 130$ .

The examination of tissue that has been fixed and mounted—and at present it is not possible to make an adequate examination of tissue prepared by any other method—is necessarily the observation of cells at one definite period of their existence. It is comparable with the examination of a unit section of cinematograph film with an attempt to interpret the preceding and succeeding events from the one picture.

The problem is that of measuring the relationship of the various cells to each other. We have only the relative positions of the cells and their morphology, and what is required in addition is the time

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5. Malassez, L. C.: *Arch. de physiol. norm. et path.*, 1885, p. 378; quoted from Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1928; Malassez, L. C., and Galippe, M. L. V.: *Les débris épithéliaux paradentaires*, Paris, Masson & Cie, 1910.

relationship, so that a proper interpretation of the sequence of events may be established. In this problem is contained that of the direction in which the change in cell structure occurs. Here somewhat arbitrary criteria are used, though continued observations support their use. Thus where relatively undifferentiated cells are present together with differentiated varieties, it is assumed, unless there is definite evidence to the contrary, that the differentiation is proceeding in a manner similar to that observed in the adult body, i. e., from the undifferentiated to the more differentiated type. Also when neighboring cysts possess similar structure, a differentiated portion of the wall of one may reasonably be regarded as a development from the preponderating tissue.

With these difficulties of interpretation in mind, we may now proceed to the interpretation of our observations. As stated previously, that well formed teeth may be present in a region in which there is well formed squamous epithelium is a common discovery. From the considerations discussed, these examples are of little value in helping us to determine the mode of origin of the teeth. The presence of the squamous epithelium at a late stage does not prove that the cellular changes have been identical with those found in other parts of the body. We have a stationary picture from which it is possible to arrive at a knowledge of the changes occurring in the cells only by a correlation of various stages to be found by the study of numerous examples. It is essential to examine early specimens. Such a one is here submitted.

The greater part of the cyst was lined by neuroglial tissue, in some parts with and in other places without a pseudostratified (? ependymal) layer of epithelium. The teratoma frequently is composed principally of nervous tissue.

The additional reasons for assuming that this is the primary form of tissue in the cyst are:

1. It is the most undifferentiated tissue present.
2. Many neighboring cysts have similar walls.
3. The tissue occupies the greater part of the wall of the cyst.

The first significant observation is the development, from the nervous epithelium, of "mucous" glands (fig. 3). Such epithelium would be usually described as "respiratory," but here it is continuous with nervous tissue. It is not necessary to emphasize this feature here, since it has been dealt with by Budde<sup>6</sup> in his discussion of a sacral teratoma. That this epithelium which is producing the glands is of the same type as that lining the greater part of the cyst, owing to some alteration in its form and not to a junction of different kinds of epithelium, is shown

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6. Budde, M.: Beitr. z. path. Anat. u. z. allg. Path. **68**:512, 1921.

by the observations that they merge gradually one into the other. Moreover, similar glands have been described as occurring in typical nervous tissue in the adult.

In the depths of the crypt described (corresponding to a dental groove), there is, for a short distance, a small area of squamous epithelium. From the area of squamous epithelium to the region of the tooth, the interpretation is apparently easy, since here we have the growing down from the epithelium of cells of definite form toward the piece of bone tissue, and here differentiation in conjunction with that of some of the connective tissue occurs in a definite manner to form a tooth.

If we use the terminology of the old hypothesis, in this small cyst we have brain tissue becoming converted into, or at least directly continuous with, respiratory tract and this in turn joining a dental groove from which a tooth is formed. Why should brain (ectoderm) and respiratory tract (entoderm) occur in continuity unless it is in the region of the neurenteric canal and if they represent this region, why is there development of teeth?

Even if we should ignore the "mucous" glands or appreciate that they arise from nervous tissue, why should the squamous epithelium of the dental groove develop from nervous tissue? Both are ectodermal, but such development suggests the differentiation of cells under the control of some unusual stimulus, rather than an attempt at formation of an embryo.

The reason for the development cannot be supplied in the present state of our knowledge. We know that a potent factor in the differentiation of tissues is their interrelationship with each other. The development of the lens from the epithelium lying above the optic vesicle, even when this is transplanted to the posterior part of the body, is a well known example. The formation of muscle in the tissue lying beneath epithelium of certain types is well substantiated. The presence of the bone in the neighborhood of the cyst in the present case may possibly be a factor in the causation of the epithelial downgrowth. That it is not the only one is shown by the presence of bone in other parts without formation of teeth. On the other hand, the development of the bone may be secondary to the epithelial development. The simultaneous development of teeth from two separate cysts in relationship to one bony mass, in one case, suggests indeed the former sequence of development as the more probable.

The important feature in this case, however, is the development of the tooth from "nervous" tissue. In the sections, the tooth appears to arise from a very small area of squamous epithelium; i. e., there is a small area of squamous epithelium at the site of origin of the dental epithelium. As pointed out, however, the present occurrence of

squamous epithelium does not necessitate the presence of this tissue at the time of origin of the downgrowth of the tooth. In fact, the small amount of this tissue, as shown by serial sections, suggests that it is a recent occurrence—there is evidence that squamous epithelium in “dermoid” cysts readily replaced other epithelia and developed concomitantly with the growth of the tooth. In any case, the tooth has developed directly or indirectly from nervous tissue.

If the tooth has developed from nervous tissue, still another problem arises. Is the “nervous” tissue really of this type, or is our interpretation inaccurate? We have only the morphology of the tissue to depend on, but this appears to be indisputable. In any case, should it be a modification of connective tissue, a great deal of the evidence for the old hypothesis disappears. The nervous or neuroglial nature of this tissue will be accepted for the present.

There is, therefore, in this example, an interrelationship of cells and tissues which is different from that seen in ordinary development. It seems certain that many of the cells of the body possess capabilities for the formation of tissues of other types which are not usually manifested. The exhibition of these potentialities presupposes that stimuli are governing cells in a manner different from that in which they are governed normally.

Further consideration of observations reveals other difficulties. As stated, teeth are usually said to be present in a “mouth” and other structures e. g., “limbs,” to be situated on the surface of the “embryo.” Many examples of teratoma, however, are multilocular, e. g., those of Shattock and Pye-Smith.<sup>7</sup> If attention is directed to one loculus, how are the remainder to be explained? Shattock’s suggestion of twin and triple teratomas only adds to our hypotheses—and to our difficulties. In the case described, the developing tooth is in relationship to one of many cysts that do not communicate with the surface of the “rudiment.” The small cyst cannot thus represent a mouth.

When we turn to general biology for assistance, we find some illuminating observations. The hypothesis of the interdependence of tissue growths is supported by numerous examples. Not only has this been found in ordinary biologic investigation, but the same phenomenon has been demonstrated recently by Huggins,<sup>8</sup> who implanted epithelium from the bladder, ureter and renal pelvis in fibrous areas of the body with subsequent formation of bone in the region of the proliferating epithelium. We have confirmed this experimental result.

Further relevant information is to be obtained from Murray’s work<sup>9</sup> on the transplantation of the blastoderm of the chick. He grafted

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7. Pye-Smith, P. H.: *Tr. Path. Soc., London* **37**:499, 1886.

8. Huggins, C. B.: *Arch. Surg.* **22**:377, 1931.

9. Murray, P. D. F., and Selby, D.: *J. Exper. Biol.* **7**:404, 1930.

the entire blastoderm and also portions into the same or other eggs. He found that in the case of the entire blastoderm, the degree of histologic differentiation was comparable with that found in normal chicks of an equivalent age, but in the case of partial grafts the degree of differentiation fell short of this. His original paper should be referred to.

In another paper,<sup>10</sup> one observation is of particular importance. Murray noted during the differentiation of this tissue a considerable number of accumulations of cells, more or less resembling the developing organs, but having no counterpart in the normal developing organism, and he referred to these as "pseudo-organs." If we should refer, in our descriptions and discussions of the complex developments in these teratomatous growths, to pseudo-organs—if we must refer to organs at all—we should be much nearer the truth and therefore considerably nearer an understanding of these growths.

In still another paper,<sup>11</sup> Murray gave drawings of his grafts that, in the absence of the knowledge of the grafting, would be accepted as pictures of teratoma. The developing graft forms as a nodule of tissue showing considerable differentiation in various directions occurring in one portion of the wall of a cyst ("vesicle"). Small cavities that he described as "skin vesicles" and "gut" and nodules of cartilage are to be found in the nodule of tissue. Since the grafts were taken from the paravertebral tissue behind formed somites of the chick, it is apparent that complicated structures may result from the development of tissue away from the vertebral region.

How we are to apply such observations to the development of teratoma is still obscure, but it indicates an avenue of research. Budde,<sup>6</sup> in an epoch-making paper, gives reasons for considering that the teratoma is derived, not from an ovum or blastomere, but from a portion of the primitive streak. The suggestions derived from experimental biology indicate that probably it will not be necessary to go back, in the development of the individual, even as far as this for the tumor anlage.

#### SUMMARY

The early development of teeth in a dermoid cyst is described and illustrated. They are shown to develop, in some cases, from primitive nervous tissue. The development of a tooth from the epithelium of a closed cyst and from tissue which, in the normal body, has no direct relationship to it, indicates that its development is the result of differentiation in tumor tissue which properly is not comparable with the growth of an embryo.

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10. Murray, P. D. F., and Huxley, J. S.: *Brit. J. Exper. Biol.* **3**:9, 1925.

11. Murray, P. D. F.: *Australian J. Exper. Biol. & M. Sc.* **5**:237, 1928.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## II. EFFECT OF CHLOROFORM ON THE NORMAL LIVER AND ON THE RESTORED LIVER FOLLOWING PARTIAL REMOVAL

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The specific action of chloroform on hepatic tissue is well known although the mode of its action is still undetermined. Chloroform induces central necrosis of the lobules of the liver in animals whether administration is by inhalation, mouth or subcutaneous injection. Similar lesions have been demonstrated in man after anesthesia with chloroform.

The remarkable regenerative powers of the liver have been demonstrated by many workers. From two thirds to three fourths of the liver may be diseased or destroyed by toxic substances, and yet it will recover its normal size and weight in a surprisingly short time. Whipple and Sperry<sup>1</sup> showed that lesions in the liver due to chloroform were completely repaired in eleven days when as much as two thirds of the organ was involved. Whipple<sup>2</sup> also showed that puppies during the first three weeks of life are less susceptible to injury by chloroform than mature dogs, and he attributed this to a protective mechanism associated with the blood-forming islands which occur in the sinusoids of the liver in newborn animals.

Since the liver of the very young animal is more resistant to chloroform, I was interested to know whether the lobe of the liver of the white rat that increases so rapidly following partial hepatectomy, displaying embryonic or fetal tendencies, is any more or less resistant to chloroform than the liver of the normal animal.

### METHODS OF INVESTIGATION

Only healthy rats, aged from 3 to 6 months and weighing from about 100 to 225 Gm., were used. The central and left lobes, comprising about 65 per cent of the entire liver, were removed according to the method of Higgins and Anderson.<sup>3</sup> Six weeks later, chloroform was administered. Histologic studies were made of the development and the repair of the hepatic lesions following the administration of

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1. Whipple, G. H., and Sperry, J. A.: *Bull. Johns Hopkins Hosp.* **20**:278, 1909.

2. Whipple, G. H.: *J. Exper. Med.* **15**:259, 1912.

3. Higgins, G. M., and Anderson, R. M.: *Arch. Path.* **12**:186, 1931.

chloroform in both normal and recently restored hepatic tissue. Only chloroform, U. S. P., "for anesthesia," was used.

The administration of chloroform by inhalation resulted in such slight and in most cases negligible lesions of the liver that the method was abandoned. On the other hand, the introduction of chloroform and liquid petrolatum in equal parts into the stomach produced such severe injury that the rats died in a few hours. Hemorrhagic lesions of the peritoneum, pleura, intestines, stomach and kidneys occurred. Bloody fluid was found in the pleural cavities and in the peritoneum. Gross hematuria was noted within a few minutes. A single subcutaneous injection of this mixture, however, proved to be a most efficient method for producing hepatic lesions. Central necrosis of the lobules of the liver occurred in the majority of rats so treated, and a dose that, if given by stomach, killed the rats in a few hours could in this way be safely administered.

*Effect of Chloroform on the Normal Liver and on the Restored Liver After Partial Removal*

Livers of Normal Rats				Restored Livers			
Weight of Rat, Gm.	Dose of Equal Parts of Chloroform and Oil, Cc.	Time Killed, Hours After Injection	Hepatic Injury*	Weight of Rat, Gm.	Dose of Equal Parts of Chloroform and Oil, Cc.	Time Killed, Hours After Injection	Hepatic Injury*
98.5	0.25	24	None	214	0.75	24	Slight
204	0.75	24	26.4	207	0.88	24	Slight
215	0.88	24	Slight	165	0.75	24	None
157.2	0.5	24	30.7	205	0.5	48	Slight
119	0.25	48	65.0	120	0.5	48	Slight
183	0.5	48	31.0	205	0.75	48	12.2
102	0.5	48	42.0	96	0.5	48	9.3
106	0.5	48	44.0	160	0.5	48	25.0
144	0.5	48	38.0	188	0.75	48	Slight
161	0.75	48	34.5	182	0.75	48	16.4
95	0.5	72	None	223	0.88	48	Slight
125	0.5	72	10.4	153	0.75	48	None
145	0.5	72	None	119	0.5	72	48.4
156	0.5	72	56.4	153	0.5	72	Slight
97	0.25	72	24.3	158	0.5	72	11.3
140	0.5	72	10.4	204	0.75	96	8.3
140	0.5	96	20.8	206	0.88	96	None
103	0.5	96	20.8	206	0.88	96	None
201	0.88	96	Slight	197	0.88	96	None
				124	0.75	96	None

\* Figures indicate percentage of hepatic tissue that was necrotic.

A method of subcutaneous administration was devised that prevented leakage and insured accurate dosage. The needle was inserted through the abdominal skin into the muscular layer, then directed forward through the muscle for a distance of 1 cm. and then superficially into the subcutaneous tissues, where the mixture of chloroform and oil was deposited. The needle was withdrawn, and the pressure of the mixture obliterated the passageway of the needle through the muscle and thus prevented loss of the chloroform.

The amounts of the mixture of equal parts of chloroform and liquid petrolatum used were as follows: for rats weighing less than 100 Gm., 0.25 cc.; for rats weighing from 100 to 150 Gm., 0.5 cc.; for rats weighing from 150 to 200 Gm., 0.75 cc., and for rats weighing more than 200 Gm., 0.88 cc.

Sixty-eight rats not operated on were given subcutaneous injections. Of these, twenty-two (33.9 per cent) died within the first four days. All had definite lesions in the liver.

Fifty-four rats in which partial hepatectomy had been done six weeks before were given similar injections. Ten of these (19 per cent) died within the first five days, and two died on the fifth day.

The surviving rats were killed at daily intervals after injection up to the twelfth day. Some of the rats killed the first three or four days might have died if they had been allowed to survive, so the percentages stated are not absolute figures for mortality; nevertheless they indicate that more of the normal rats died following the injection of chloroform than rats in which partial hepatectomy had been done.

Histologic studies were carried out on the livers of all the rats that were killed and of a few of those that died in which autolysis was not present. The following staining mediums were used: hematoxylin and eosin; sudan III; Mallory's connective tissue stain; van Gieson's connective tissue stain; del Rio Hortega's gold chloride ammoniacal silver carbonate, and del Rio Hortega's ammoniacal silver carbonate cold for mitochondria.

#### OBSERVATIONS

The central necrosis that occurred in the lobule of the liver after the subcutaneous injection of chloroform was more or less alike in both the normal and the newly restored liver except in extent. Necrosis following comparable injections was always far less in the restored liver than in the normal liver, but the description that follows is applicable to either of the two types of livers.

Six hours after an injection of chloroform there was slight microscopic evidence of chloroform injury, although grossly the liver appeared entirely normal. These slight changes involved distention of the sinusoids and occasionally disruption of the endothelium of the central veins, and there was a definite increase of fat in the paler staining central areas. Definite necrosis about the central veins occurred eighteen hours after the injection. The sinusoids were obliterated in these areas of necrosis, owing essentially to the swelling of the cells, and the endothelium of the central veins invariably showed disruption. A marked deposit of fat occurred in the necrotic areas as well as in the periphery of these zones where there was no definite necrosis.

Twenty-four hours after the injection, necrosis was marked. The cells were swollen, and the nuclei were pyknotic, small and often absent. Many cells had fragmented, and wandering mononuclear cells had invaded the necrotic zones to remove dead cells and other débris. Sudan III stains showed marked fatty degeneration, and necrotic cells were more hyaline and did not stain with any of the staining technics employed. At the periphery of the lesion where fatty changes were more marked, the cells were extensively vacuolated.

Marked central necrosis occurred forty-eight hours after the injection (figs. 1 and 2). The hepatic reticulum, however, still persisted and was apparently unaffected by the chloroform. Mitotic figures were abundant at forty-eight hours after the injection, and as many as ten or twelve figures were often identified in one field. They were usually situated just peripheral to the areas of fatty change.



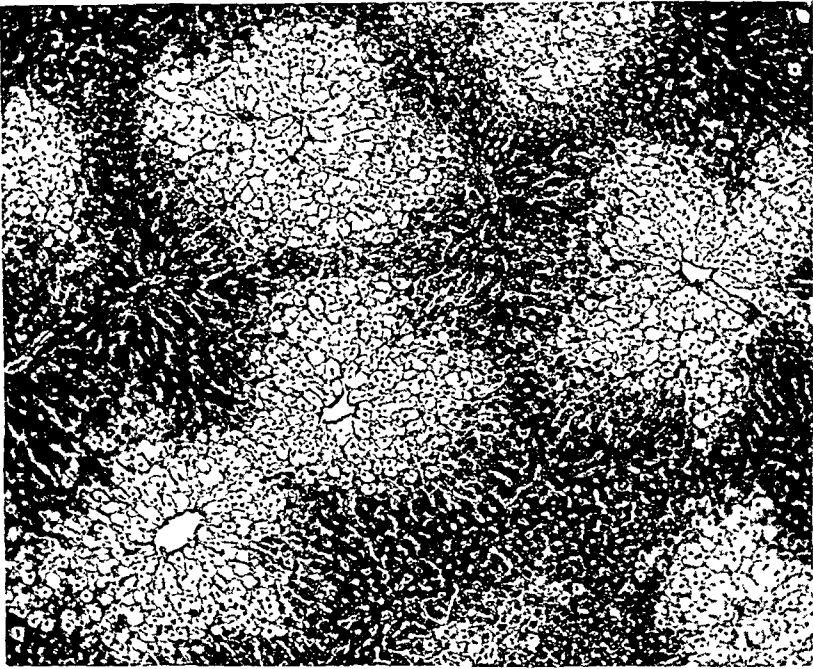


Fig. 1.—Liver of a normal rat forty-eight hours after injection of chloroform (del Rio Hortega technic, cold method;  $\times 60$ ).

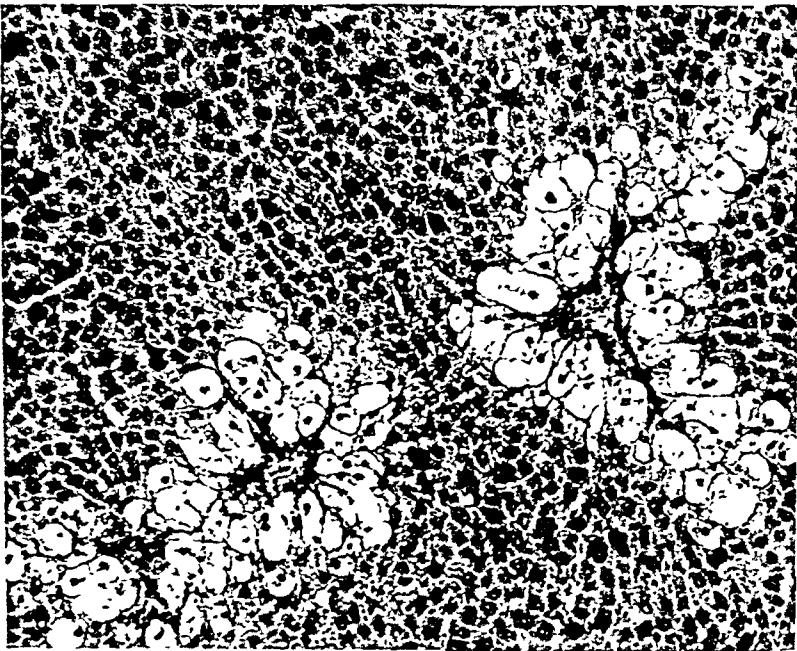


Fig. 2.—Restored liver of a rat forty-eight hours after injection of chloroform ( $\times 125$ ).

As soon as necrosis had begun, phagocytic cells entered the necrotic areas. They were mainly wandering monocytic cells, but there were some polymorphonuclear leukocytes. This cellular response was first observed twenty-four hours after the injection of chloroform, and it became marked on the second day, attaining its height on the third day (fig. 3). Giant cells, which have been reported by some observers of chloroform poisoning in other laboratory animals, were not seen in the rat. When the removal of necrotic cells and material from the central areas continued, mitosis progressed vigorously, and new cells pushed into the areas that were formerly necrotic. The portal spaces were never

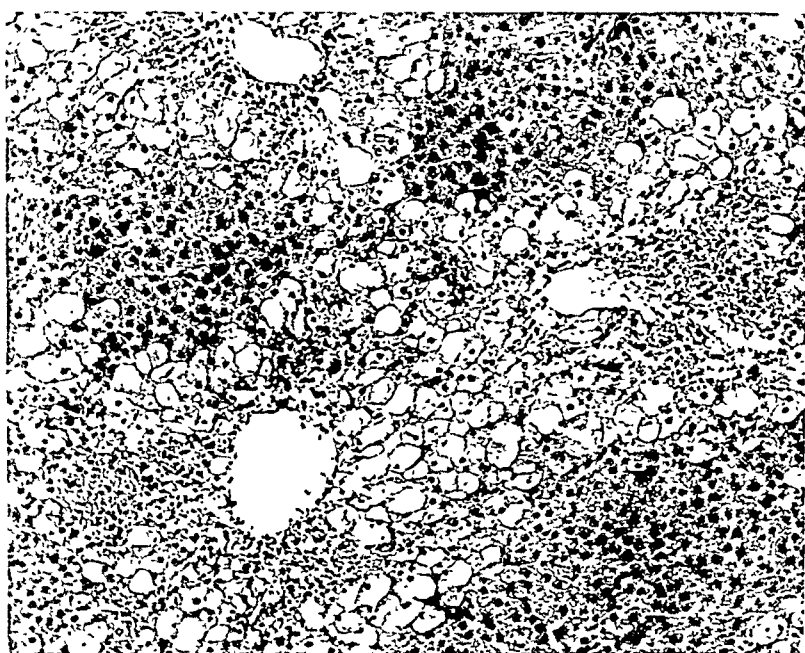


Fig. 3.—Liver of a normal rat seventy-two hours after injection of chloroform. Repair is in progress; infiltration by phagocytic cells may be observed ( $\times 100$ ).

involved, and cytologic changes were not observed in the portal spaces or in the cells surrounding them.

On the fourth day, the extent of the lesion was appreciably less. Phagocytic cells were still present, but the greater part of the necrotic material had been removed, and the necrotic areas were filled to a great extent by new cells, which had crowded in from the adjacent normal areas. Reticulum may play a part in the repair of the lesion, for the ammoniacal silver carbonate stains showed that these fibers persisted during the third and fourth days when the greater part of the necrotic material had been removed. Even the finest fibrils were uninjured, and it is probable that the reticulum proved a framework along which the new hepatic cells migrated into the formerly injured areas.

On the fifth day, the repair was complete, and the necrotic materials had been removed (fig. 4). The endothelium of the central veins was often incomplete, and the sinusoids were quite irregular; but new hepatic cells occupied the central spaces that had formerly contained the necrotic material.

The literature on the form and distribution of mitochondria in the animal cells is enormous, and no attempt will be made to review it here except to quote Cowdry's definition.<sup>4</sup> Cowdry assembled and reviewed more than five hundred papers on mitochondria and defined mitochondria as follows: "Substances which occur in the form of granules, rods

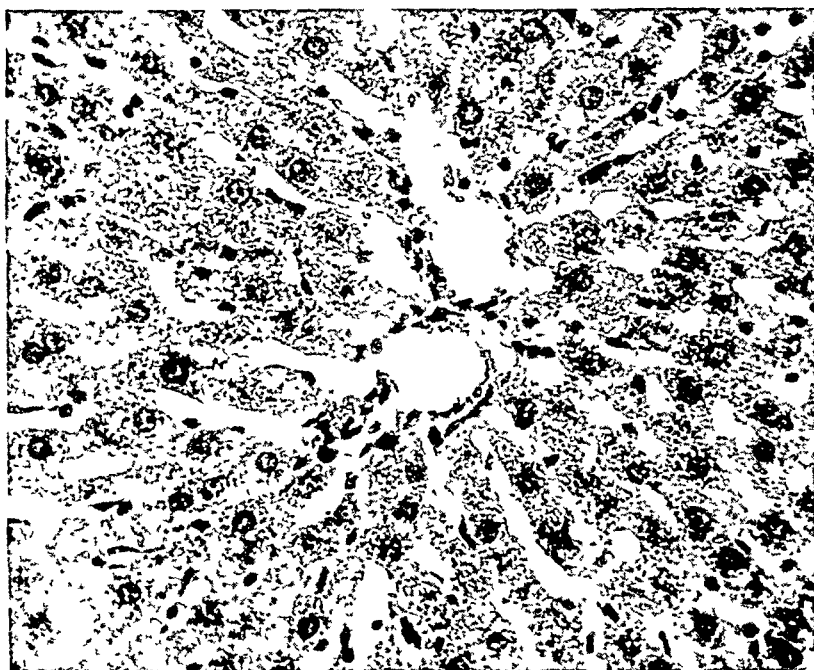


Fig. 4.—Liver of a rat on the fifth day after injection of chloroform. Repair of the lesion is essentially complete ( $\times 250$ ).

and filaments in almost all living cells, which react positively to Janus green B and which by their staining reactions and solubilities resemble phospholipins and to a lesser extent albumins." Cowdry regarded them as the most delicate indicators of cellular injury. The del Rio Hortega ammoniacal silver carbonate cold method for mitochondria was selected as the most reliable of the several methods that were employed.

Normally the mitochondria of the hepatic cell are fine rods and fine granules scattered rather uniformly throughout the cell, but immediately following the absorption of chloroform, they change from rods and small granules to coarse spherical granules (figs. 5 and 6). This demon-

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4. Cowdry, E. V.: *General Cytology*, Chicago, University of Chicago Press, 1924, p. 313.

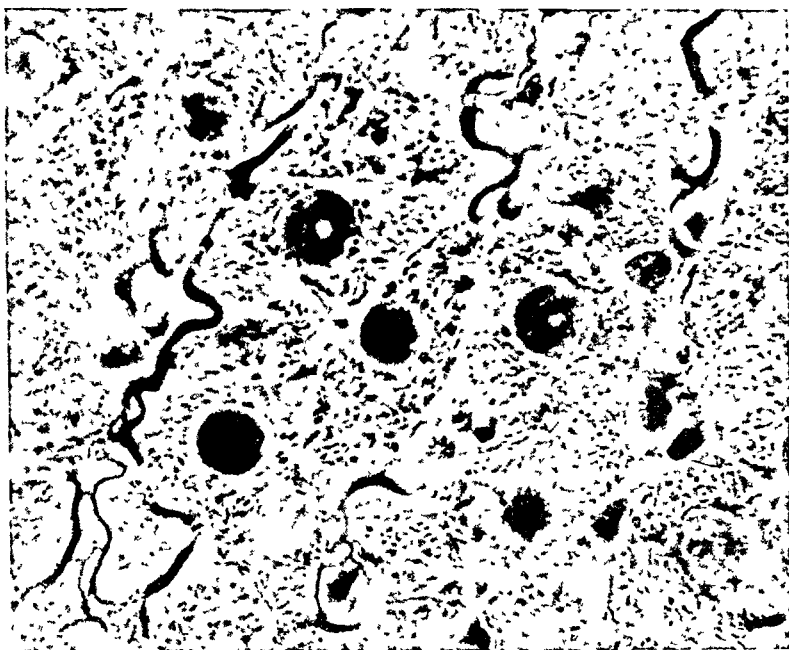


Fig. 5.—Mitochondria in the liver of a rat (del Rio Hortega technic, cold method;  $\times 1,250$ ).

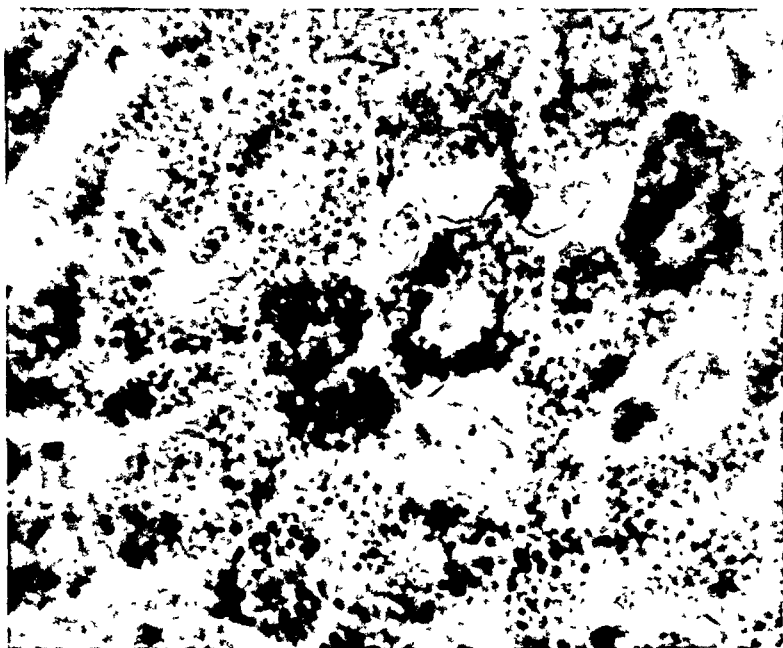


Fig. 6.—Mitochondria in the liver of a rat (del Rio Hortega technic, cold method;  $\times 1,250$ ).

strates their delicate and immediate reaction to chloroform. This change in shape of the mitochondria was not limited to the central areas of necrosis, but was uniform throughout the lobules. Twenty-four hours after the administration of chloroform, however, the mitochondria were normal again, appearing as fine rods and fine granules, and could not be distinguished from the mitochondria of a liver that had not been exposed to the action of chloroform.

The extent of the lesion in both the normal and the restored livers was determined by the use of a projectoscope of the Eddinger type. Six representative fields from the microscopic sections obtained from each rat in which the hepatic lesion was definite enough for accurate determination were projected on uniform white cardboard. The outlines of the necrotic areas were traced and cut out, and their weight was compared with the weight of the entire tracing. This gave an approximate estimate of the percentage of the lobule that had sustained necrosis. The data, which have been condensed into table 1, rather definitely show that the extent of the lesion was far greater in the normal rats not operated on than in those possessing a newly restored organ following partial removal. This suggests that the liver that is restored so rapidly following operation may possess such protective agencies as to make it comparable to the hepatic organ in the new-born rat and thus less susceptible to chloroform.

#### COMMENT

Chloroform induced a specific destructive lesion in hepatic tissue and also profound lesions in the heart and kidneys. Large doses caused death before a demonstrable hepatic lesion had time to occur. Mitochondrial stains indicated that the chloroform affected the hepatic cell within a few minutes of the time of its administration. The lesion produced in the liver by chloroform was a central necrosis associated with fatty changes. In some cases, as much as from two thirds to three fourths of the liver was destroyed by chloroform, and yet the animals survived and entirely recovered.

The extent of the lesion varied with the method of administration. When chloroform was administered by inhalation to either normal rats or those with recently restored liver after partial hepatectomy, slight, negligible lesions occurred. This probably illustrates the protective action of a well balanced diet. When chloroform was administered by stomach, such severe lesions occurred that the rats did not survive through the day of injection, in spite of the fact that the doses administered were no greater than those given by the subcutaneous method. Marked hemorrhagic lesions were present in the pleura, peritoneum, stomach, intestines, liver, kidneys and spleen. The coagulation factors of the blood were altered, and the peritoneum and pleural cavities contained large amounts of hemorrhagic fluid. Hematuria also occurred.

The subcutaneous injection of chloroform and sterile liquid petrolatum in equal amounts proved excellent. In some cases, the lesion was slight, involving only a few cells about the central vein and disruption of the endothelium of the vein with widening of the sinusoids due to congestion; in other cases, it was so severe as to involve as much as two thirds of the lobule of the liver. The extent of the lesion was not always proportional to the dose of chloroform administered; a stated dose given to a small rat did not in some cases produce as severe a lesion as the same dose given to a larger rat. Other factors also entered.

Evidence of necrosis was sometimes found at eighteen hours, but it was never extensive, whereas at twenty-four hours the lesion was always well defined. The lesion was most marked, however, on the second and third days after the administration of chloroform. Repair of or regeneration of the liver, as indicated by mitosis, occurred as early as twenty-four hours after the injection of chloroform, but it, too, was marked at forty-eight and seventy-two hours. Mitotic figures were most abundant in normal hepatic cells in the periphery of the lobule between the area of fatty changes and the portal spaces.

Repair was aided by the wandering phagocytic cells, which largely removed the necrotic tissue by the fourth day. At the fifth day, repair, it may be said, was 95 per cent complete. It was entirely complete by the sixth or the seventh day, when the endothelium of the central veins had been reestablished, and fatty changes had disappeared. Van Gieson's stain and Mallory's connective tissue stain indicated no activity of fibroblastic elements in the repair of the lesion produced by chloroform.

Repair of the lesion produced by chloroform took place in recently restored hepatic tissue exactly as in normal hepatic tissue. Since the lesion was less extensive in recently restored hepatic tissue, complete repair was more rapid in the rats that had been partially hepatectomized than in the normal rats. This probably was due to the lesser extent of the lesion and not to any greater power of repair on the part of the recently restored hepatic tissue.

A comparison of the two groups of rats from the standpoint of the extent of the lesions induced and the mortality encountered leads one to conclude that recently restored hepatic tissue is either more resistant or perhaps less susceptible to the effects of chloroform than normal hepatic tissue.

#### SUMMARY

Normal rats and those with recently restored hepatic tissue after partial hepatectomy, when maintained on standard rations, were very resistant to the action of chloroform administered by the inhalation method, but were rapidly killed by chloroform administered by stomach tube.

Injection of chloroform and sterile liquid petrolatum, in equal parts, into the subcutaneous tissues of the abdominal wall proved an easy and accurate method of administration, although the lesion produced was not invariably proportional to the dose. In some cases, a small rat did not have as extensive a lesion as a larger rat receiving the same dose.

Central necrosis occurred on the first day, but was much more marked on the second and third days after the injection. In the normal rat, repair was almost complete at the fifth day. There was no scar tissue. The lesion was far less extensive in the newly restored liver following partial removal than in the normal liver, and repair was more rapidly complete in the animals operated on than in those with intact livers. This may have been due to the lesion being less extensive and not to greater reparative power.

When the extent of necrosis was determined by graphic methods, normal rats showed a much higher percentage of necrosis than rats with a recently restored organ.

A larger percentage of normal rats died (33.9 per cent) after subcutaneous injection of chloroform than rats with recently restored hepatic tissue (19.2 per cent). These data lead one to conclude that recently restored hepatic tissue is more resistant or less susceptible to chloroform than the liver of normal rats.

# NORMAL FAT CONTENT OF THE KUPFFER CELLS

## HISTOLOGIC STUDY

VICTOR LEVINE, M.D.

CHICAGO

The so-called Kupffer cells of the liver were first described in 1869 by Ponfick,<sup>1</sup> who discovered them as stained elements attached to the walls of the hepatic sinusoids, by injecting mercuric sulphide and ultramarine blue. From that day to this the phagocytic abilities of the cells have never been in doubt. There has, however, been considerable dispute as to the exact nature and origin of the cells. Von Kupffer<sup>2</sup> in his first work in 1876 believed the cells to be perivascular, not endothelial. By the time of his last paper, in 1899,<sup>3</sup> he had completely changed his opinion and considered them to be purely endothelial. Since then their endothelial, or reticulo-endothelial, nature has not been in serious dispute.

There is still disagreement as to whether or not all the cells lining the sinusoids, the endothelial cells, are in fact Kupffer cells which can assume phagocytic properties. Von Kupffer's<sup>3</sup> final opinion was that they were the only endothelial cells of the capillaries. Schilling<sup>4</sup> believed that it was impossible to differentiate Kupffer cells and endothelial cells histologically, and that all actively phagocytosing Kupffer cells develop from preexisting endothelial lining. Nathan,<sup>5</sup> studying human embryos, concluded that the evolution of the hepatic endothelium was the same as that of all reticular apparatus, and that the Kupffer cells were the only vascular endothelium. Maximow<sup>6</sup> thought that practically all the cytologic elements of the capillary wall might assume the properties of Kupffer cells. Higgins and Murphy<sup>7</sup> and Higgins and Mann,<sup>8</sup> using injections of graphite suspensions, came to the conclusion that not all the endothelial elements are capable of phagocytosis. On the

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From the Department of Pathology, Cook County Hospital, R. H. Jaffé, Director.

1. Ponfick, E.: *Arch. f. path. Anat. u. Physiol.* **48**:1, 1869.
2. von Kupffer, C.: *Arch. f. mikr. Anat.* **12**:353, 1876.
3. von Kupffer, C.: *Arch. f. mikr. Anat.* **54**:254, 1899.
4. Schilling, V.: *Centralbl. f. allg. Path. u. path. Anat.* **19**:577, 1908.
5. Nathan, M.: *La cellule de Kupffer*, Thèse de Paris, no. 118, 1908.
6. Maximow, A. A.: *The Macrophages or Histiocytes*, in Cowdry, E. V.: *Special Cytology*, New York, Paul B. Hoeber, Inc., 1928, pp. 425-484.
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basis of experimental work, Pfuhl<sup>9</sup> divided the Kupffer cells into the seizing, projecting type, and the flat, digesting type. As the result of studies on human material, Schilling<sup>4</sup> made the statement that the flat endothelial cells were resting cells which became much swollen during phagocytic activity and later, when completely saturated with the engulfed particles, assumed an intermediate, oval form.

Nathan<sup>5</sup> divided the functions of the Kupffer cells into three groups: phagocytosis of large elements such as blood cells or cell fragments, phagocytosis of bacteria and phagocytosis of granules, including fat granules. Von Platen<sup>10</sup> in 1878 was the first to report phagocytosis of fat. Soon afterward various workers found carmine, erythrocytes, leukocytes, colloidal silver and other substances as inclusions within Kupffer cells. Schilling<sup>4</sup> was the first to describe phagocytosis of bacteria in detail. More recently Aschoff and Kiyono,<sup>11</sup> on the basis of intravital staining with lithium carmine, definitely considered Kupffer cells as a part of the reticulo-endothelial system.

Gilbert and Carnot<sup>12</sup> made experiments with the injection of fat and found the fat at first in the Kupffer cells and only after some hours in the hepatic cells. They inferred that the Kupffer cells passed the fat to the hepatic cells. Nathan<sup>5</sup> injected stearic acid and linseed oil and found inclusions of fat particles in the Kupffer cells. Saxl and Donath<sup>13</sup> injected a fine fat emulsion and after watching its disappearance from the blood reported their results as a basis for a functional test of the reticulo-endothelial system. That it is not such a test was shown in the experiments of Jaffé and Berman,<sup>14</sup> who, working with the same emulsion, found that the fat was taken up almost immediately by the Kupffer cells of the liver and not to any marked extent by the reticulo-endothelium elsewhere. These investigators also stated that the fat droplets were passed quickly to the hepatic cells from the Kupffer cells, especially in the peripheral acinar zones. Thus they felt that the injection of this particular emulsion tests chiefly the hepatic function, particularly the relations between hepatic and Kupffer cells, and that its use is in no sense a test of function for reticulo-endothelium in general. In this regard it may also be mentioned that Sacks<sup>15</sup> injected

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9. Pfuhl, W.: *Ztschr. f. Anat. u. Entwicklsgesch.* **81**:90, 1926.

10. von Platen, O.: *Virchows Arch. f. path. Anat.* **74**:268, 1878.

11. Aschoff, L., and Kiyono, K.: *Verhandl. d. deutsch. path. Gesellsch.* **16**:107, 1913. Kiyono, K.: *Die vitale Karminspeicherung; ein Beitrag zur Lehre von der vitalen Färbung mit besonderer Berücksichtigung der Zelldifferenzierungen im entzündeten Gewebe*, Jena, Gustav Fischer, 1914. Aschoff, L.: *Lectures on Pathology*, New York, Paul B. Hoeber, Inc., 1924, p. 7.

12. Gilbert, A., and Carnot, P., quoted by Nathan.<sup>5</sup>

13. Saxl, P., and Donath, F.: *Wien. klin. Wchnschr.* **38**:66, 1925.

14. Jaffé, R. H., and Berman, S. L.: *Arch. Path.* **5**:1020, 1928.

15. Sacks, B., quoted by Aschoff: *Lectures on Pathology*, New York, Paul B. Hoeber, Inc., 1924, p. 94.

a fine emulsion of cholesterol, and found that it was deposited in the cells of the reticulo-endothelium.

Jornier <sup>16</sup> made experiments feeding fat and observed fat in both the hepatic and the Kupffer cells in no constant proportion, although he did find it especially marked in the Kupffer cells of the peripheral acinar zones. He found that the fat first increased in amount in the hepatic cells and then began to decrease from the eleventh hour after feeding, at which time the amount of fat in the Kupffer cells started increasing. This would seem to indicate that the fat passed in the reverse direction, from the hepatic to the Kupffer cells. More work on this type of experiment would seem to be necessary to determine the correctness of these results, and to explain the great difference in the findings of the two types of experimentation.

Little has been written about the presence of fat in the Kupffer cells in pathologic conditions of human beings. Schilling <sup>4</sup> mentioned a case of lipemia and pernicious anemia in which both fat and iron were found in the Kupffer cells, but only iron in the hepatic cells. He concluded that the lipemia was of short duration, and that the fat was actively removed from the blood by phagocytosis. In his experimental work, however, he found fat in varying proportions in hepatic and Kupffer cells, and believed that the same poison that caused fatty changes in the hepatic cells also caused the Kupffer cells to take up fat. Nathan <sup>5</sup> mentioned the findings in many different pathologic conditions. In connection with only one case, however, does he speak of fat in the Kupffer cells. This was a case of eclampsia in which both mother and child had marked swelling of the Kupffer cells, which were stuffed with fat.

Fischer <sup>17</sup> studied the localization of fat in the livers of 150 patients who died from natural causes. The livers of new-born infants and livers with gross destructive lesions, such as abscesses or cirrhosis, were excluded. None of the 150 livers was free from fat. He found no correlation between the state of bodily nutrition and the amount of fat in the liver. He also concluded that the length of time between death and postmortem examination had no influence on the amount of fat in the liver. In 3 of these 150 cases, fat was found solely in the Kupffer cells. In 43 cases, there was no fat in the Kupffer cells; in 59 cases, they were all filled with fat. In a similar series of 150 human livers, Helly <sup>18</sup> found fat in the Kupffer cells of 40 per cent. Neither Fischer nor Helly could discover any relation between the amount of fat in the Kupffer cells and the amount or localization of fat in the hepatic cells. Fischer also stated that there was no relation between the

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16. Jornier, quoted by Nathan.<sup>5</sup>

17. Fischer, W.: *Virchows Arch. f. path. Anat.* **208**:1, 1912.

18. Helly, K.: *Beitr. z. path. Anat. u. z. allg. Path.* **51**:462, 1911.

cause of death and the amount of fat in the Kupffer cells, and considered that the fat in the Kupffer cells was not characteristic of an infectious process, since it was completely absent in several patients dying of infections, such as pneumonia or peritonitis. In livers with definite stasis, he found fat more frequently present in the Kupffer cells than in the hepatic cells. He granted the usual presence of fat in the Kupffer cells of the gravid liver, and he was in accordance with Rössle<sup>19</sup> on findings in the diabetic liver.

Rössle<sup>19</sup> observed that in the diabetic liver the Kupffer cells were almost always stuffed with fat. In his opinion, this, with increased pericapillary connective tissue, made the picture diagnostic of diabetes. Helly,<sup>18</sup> while agreeing as to the finding of fat in the Kupffer cells, did not consider the presence of fat as pathognomonic.

Thus no definite conclusions have been reached as to the causes of the appearance of fat in the Kupffer cells. All that can be said is that most of the workers have considered its presence as pathologic (Schilling, Nathan, Rössle).

No reference has been found to a study of the normal lipid content of the Kupffer cells or to the possibility of fat being normally present in the Kupffer cells. Fischer<sup>17</sup> left one to infer that fat may have been present in the hepatic cells before the development of the disease that caused the patient's death, but he made no direct mention of this possibility. Helly<sup>18</sup> made the statement that, while pneumonia or septic processes (as well as diabetes) seem to cause the appearance of fat in the Kupffer cells, fat is also found there in infection-free persons. He did not, however, mention the possibility that the fat in the Kupffer cells of the last-mentioned group could be a normal finding. Aschoff,<sup>15</sup> in mentioning Sack's work, stated that injected cholesterol localizes to the reticulo-endothelial system, and that this location is different from that in which fat is normally found. Kawamura<sup>20</sup> described the presence of doubly refracting lipoids in the Kupffer cells under different pathologic conditions. It has also been shown by Anitschkow<sup>21</sup> and many other investigators that in the experimental cholesteatosis produced by feeding, the Kupffer cells are always filled with cholesterol and cholesterol esters.

In order to determine the normal fat and lipid content of the Kupffer cell it was found advisable to examine the liver in persons who had met sudden, violent deaths. Forty-three livers were examined.

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19. Rössle, R.: *Verhandl. d. deutsch. Path. Gesellsch.* **10**:334, 1907.

20. Kawamura, R.: *Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose*, Jena, Gustav Fischer, 1927.

21. Anitschkow, N.: *Deutsche med. Wchnschr.* **39**:741, 1913; *Med. Klin.* **10**:465, 1914.

## METHODS

On formaldehyde-fixed material the following stains were used: sudan III and hematoxylin, Nile blue sulphate, Fischler's stain and Lorrain Smith-Dietrich stain. Unstained frozen sections were also examined for doubly refracting lipid bodies. Pieces of tissue fixed in Ciaccio solution were stained by that method.

In interpreting the results, one must bear in mind that a certain staining reaction is not proof of the presence of a substance in pure state. Since fatty and lipid substances are generally present as mixtures, the staining properties are due only to the predominance of one type (as neutral fat or fatty acid, for example). Chemical analysis reveals more lipid material than can be demonstrated by histochemical methods. Thus the procedure used here can in no sense be taken as absolutely determining the type and the amount of each of the various lipid substances, but only as an indication of the predominating kind present in the various cells.

## RESULTS

The table shows the results obtained in the 27 livers examined by all the staining methods. For completeness' sake, the findings for hepatic cells are listed as well as those for Kupffer cells.

In every liver, fat was found in varying amounts in both the hepatic and the Kupffer cells with the sudan III stain. When the Kupffer cells contained much fat and the hepatic cells little, the sudan III stain was almost specific for the Kupffer cells, which, even under low power magnification, stood out as orange-yellow dots. In only 3 cases was the amount of sudanophil material in the Kupffer cells tabulated as low as a trace. In 2 of these cases (22 and 24), brown granules were found in the hepatic cells, which leads one to assume that these livers may not have been completely normal at the time of death. The 16 additional livers not listed in this table were stained only by the sudan III and hematoxylin method. Fourteen showed results similar to those listed, while in 2 the Kupffer cells contained no fat. The latter showed early periportal cirrhosis and can be discarded from the group as abnormal.

On careful examination of the Kupffer cells, the picture closely fitted Fischer's description<sup>17</sup> in which he said that the fat droplets were smaller than the nucleus and usually were arranged in graded rows with the largest droplets next to the central nucleus and the smallest droplets farthest from the nucleus, thus giving the cells a pointed appearance. This is illustrated in the figure. The amount of fat in the hepatic cells varied considerably and bore out Fischer's contention<sup>17</sup> that it had no relation to the state of nutrition, which is listed in the table.

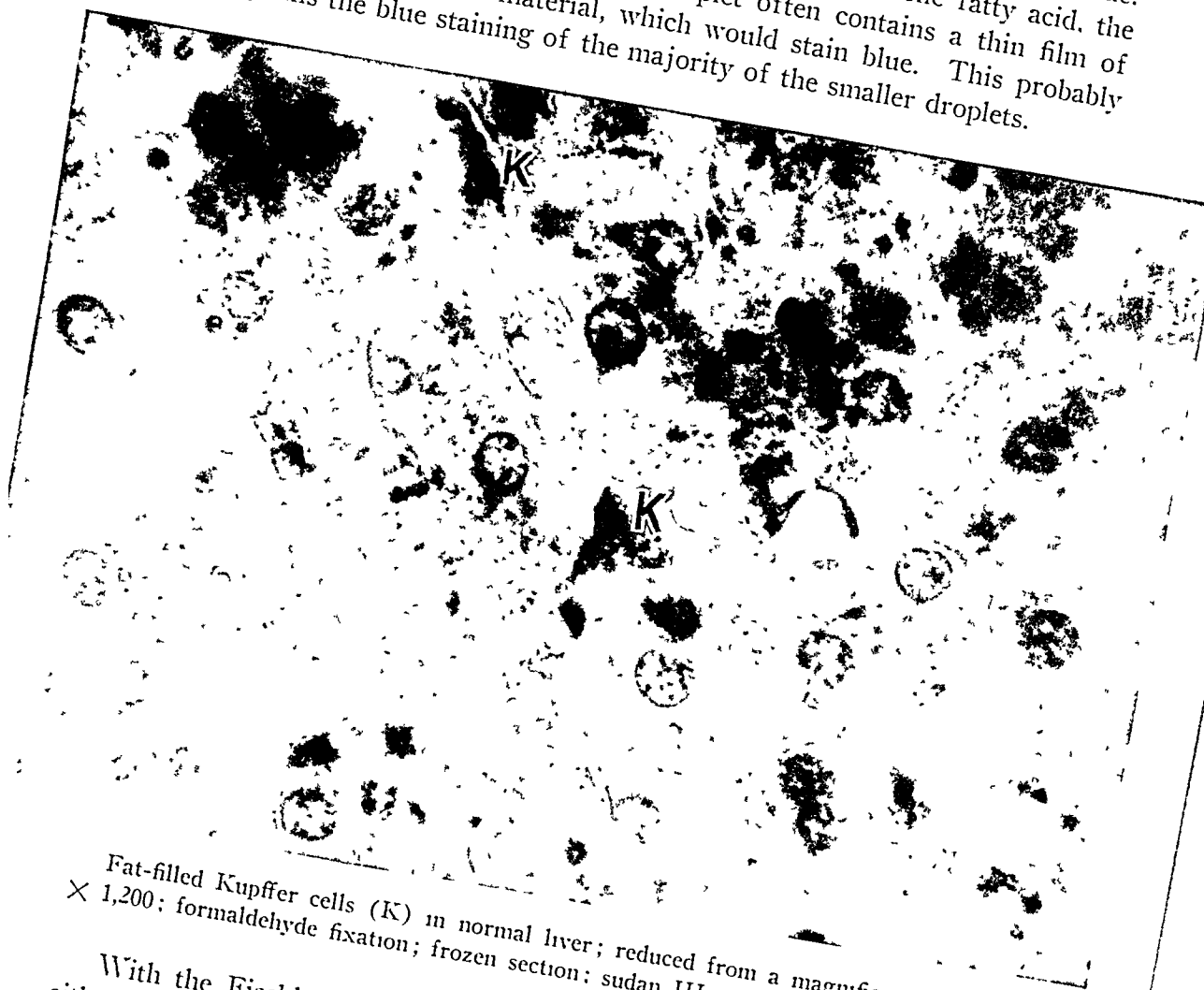
With the Nile blue sulphate stain, a variety of results was obtained. In some, all of the granules in the Kupffer cells stained blue; in some,

Case	Race	Sex	Age	Cause of Death	Time Between Injury and Death	State of Nutrition	Lipin Content of									
							Kupffer Cells				Hepatic Cells					
							Sudan III	Nile Blue Sulphate	Lorrain Smith-Dietrich Stain	Claeelo Stain	Doubly Refractile Fat	Sudan III	Nile Blue Sulphate	Lorrain Smith-Dietrich Stain	Claeelo Stain	Doubly Refractile Fat
1	W	M	50	Fight and stab wounds	Few min.	Good	+++	Blue	+	0	0	+++	Pink	Trace	0	+++
2	W	M	65	Fell from third floor	15 min.	Good	++	Blue	Trace	0	0	+	Pink	Trace	Trace	+++
3	W	M	19	Automobile accident	3 hr.	Good	+	Blue	Trace	0	0	+	Pink	0	Faint	+++
4	W	M	40	Gunshot in head	Inst. death	Good	++	Blue	++	0	0	+++	Pink and purple	+	Faint	+++
5	Mex. C	M	31	Gunshot in abdomen	3 hr.	Good	+	Blue	0	0	0	Trace	Pink	Trace	Trace	+++
6	C	M	35.5*	Stab wound in left axilla	2 hr.	Fair	++	Blue	0	0	0	+	Purple	0	Trace	+++
7	W	M	26.5	Beaten about head	Few min.	Good	++	Blue	Trace	0	0	+++	Pink	++	0	+++
8	W	M	51	Gunshot in chest	Inst. death	Good	++	Blue	+	0	0	+	Blue	+	+	+++
9	W	M	20	Gunshot in head	Inst. death	Good	+++	Blue	0	0	0	Trace	Pink	0	Trace	+++
10	W	F	20	Stab wound in heart	Inst. death	Good	+++	Pink and blue	0	0	0	+	Pink	0	+	+++
11	W	M	30	Skull fracture, hit over head	Few hr.	Good	++	Pink	0	0	0	Trace	Blue	+	+	+
12	W	M	20	Skull fracture	Few min.	Good	++	Pink and blue	0	0	0	+	Purple	+	+	++
13	W	M	72	Automobile accident, ruptured liver,	About 2 hr.	Fair	+++	Pink	Trace	Trace	0	+	Pink	+	Trace	++
14	W	M	39	Gunshot in head and ruptured lung	Inst. death	Good	+	Blue	0	0	0	+	Blue	Trace	+	+
15	W	M	28	Gunshot in head	Inst. death	Good	Trace	.....	0	0	0	+	.....	Trace	+	++
16	W	M	30	Gunshot in heart and lungs	Inst. death	Good	+	Blue	0	0	0	+	Purple	Trace	Trace	+
17	W	M	35	Gunshot in lungs	Inst. death	Fair	+++	Blue	0	0	0	+	Blue	Trace	.....	+++
18	C	M	41	Stab wound in heart	Inst. death	Good	+++	Pink	0	0	0	+++	Pink	+	Faint	+++
19	W	M	35	Gunshot in heart and lungs	Inst. death	Not good	+	Pink and blue	Trace	Faint	0	+	Pink	Trace	Trace	+
20	W	M	35	Beaten about head	Sudden death	Obese	++	Pink	Trace	Trace	0	+++	Pink	+	Faint	++
21	W	M	35	Gunshot in heart and chest	Inst. death	Good	+	Pink	Trace	Trace	0	+++	Pink and purple	+	0	+++
22	C	M	35	Gunshot in heart and lungs	Inst. death	Good	Trace	Blue	Trace	Trace	0	Trace	Purple	Trace	Trace	+
23	C	M	25	Gunshot in heart and chest	Inst. death	Good	+	Blue	Trace	Faint	0	Trace	Blue	Faint	+	++
24	C	M	20	Gunshot in lungs and aorta	Inst. death	Good	Faint	Blue	0	Faint	0	Trace	Blue	Faint	+	+
25	W	M	10	Gunshot in abdomen	3 hr.	Good	+++	Pink and blue	Trace	Trace	0	+	Pink, purple and blue	Trace	Trace	+
26	W	M	50	Gunshot in left arm with hemorrhage	About 1 hr.	Good	++	Pink	0	0	0	+	Pink	Trace	Trace	++
27	W	F	20	Gunshot in chest	About 30 min.	Good	+	Pink and blue	Faint	0	0	++	Pink and purple	Trace	0	Trace

The Fischer stain was also used, but revealed no fat droplets in either type of cell.

\* a = about.

pink, and in some, a mixture of blue and pink. The blue-staining granules predominated, however, and this was thought to be due to their smaller size. In the hepatic cells, as a general rule, the large fat droplets stained pink, the medium sized ones purple and the small ones blue. While a pink stain indicates neutral fat and a blue one fatty acid, the surrounding membrane of a fat droplet often contains a thin film of fatty acid and lipoid material, which would stain blue. This probably explains the blue staining of the majority of the smaller droplets.



Fat-filled Kupffer cells (K) in normal liver; reduced from a magnification of  $\times 1,200$ ; formaldehyde fixation; frozen section; sudan III and hematoxylin stain.

With the Fischler stain for fatty acids, no droplets were found in either Kupffer or hepatic cells that took the stain; this clarifies the findings with the Nile blue sulphate stain and practically proves that the fat is mainly neutral.

With the Lorrain Smith-Dietrich stain for lipoids, some of the Kupffer cells showed lipoid granules and some did not. No correlation seemed to exist between the amount of sudanophil material in the Kupffer cells and the presence of lipoid granules demonstrable by this method. In most cases, the hepatic cells were positive with this stain.

owing in large part to the fact that the envelop of most of the large fat droplets could be seen to contain lipoids stainable by this method.

With the Ciaccio stain for lipoids, a variety of results was also obtained. Here again there appeared to be no relationship between the findings with the usual sudan III stain and those obtained with this special sudan stain. Most of the hepatic cells had granules that took this stain, while the majority of the Kupffer cells did not. The amount of lipid found, by this stain, in the Kupffer cells was only very slight.

The examination for doubly refractile bodies disclosed none in the Kupffer cells and a varying amount always present in the hepatic cells. Double refraction of lipid material is indicative of the presence of considerable amounts of cholesterol and cholesterol esters in the lipid mixtures. Since Anitschkow<sup>21</sup> has shown that Kupffer cells store cholesterol from the blood stream, the finding probably indicates that under normal conditions the cholesterol is passed to the hepatic cells too rapidly to be demonstrated in the Kupffer cells by the method used here.

#### SUMMARY

Although Kupffer cells have been described since 1869 and definitely identified as endothelial elements since 1899, scant attention has been given to their fat content under pathologic conditions, and the question of a normal fat content has been neglected.

A study of the histologically demonstrable fat and lipid substances of both hepatic and Kupffer cells was made on the livers of 27 persons dying a sudden, violent death. The fat content alone was studied in an additional 16 similar cases. In all but 2 of the 43 cases fat was found in the Kupffer cells. In the exceptions, early cirrhosis of the liver was present. In each case, fat was also found in the hepatic cells.

Special stains demonstrated that the sudanophil material in both hepatic and Kupffer cells was mainly neutral in type, and that lipid substances were at times present in both kinds of cells. Doubly refracting lipid substances were found in the hepatic cells, but not in the Kupffer cells.

Thus one can conclude that neutral fat is normally found in the Kupffer cells of the liver.

# OCCURRENCE OF A CALCAREOUS ARTERIAL LESION IN GOITER

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Curtis<sup>1</sup> and Curtis and Delaney<sup>2</sup> have drawn attention to the occurrence of calcification of the thyroid arteries in goiter, quoting Gutknecht,<sup>3</sup> Jores,<sup>4</sup> Isenschmid,<sup>5</sup> Hesselberg<sup>6</sup> and Wangensteen.<sup>7</sup> Gutknecht observed extensive hyalinization and calcification in blood vessels in thyroid glands, including capillaries. Jores described granular calcareous deposits associated with fragmentation of the internal elastic lamella in arteries of goitrous thyroid glands. Arteries so involved also presented a thickened intima. This involvement was independent of generalized arteriosclerosis. Hesselberg and Isenschmid noted arteriosclerosis in the thyroid glands of infants, the former in a new-born infant. Wangensteen found intimal thickening and calcification and splitting of the internal elastic coat to be of not infrequent occurrence in goiters of cretins. Karsner<sup>8</sup> stated that arteriosclerosis is common in goiter, often independent of arteriosclerosis in other parts of the body.

The present study was undertaken in an attempt to determine the incidence of calcification of the internal elastic lamella in goitrous thyroid glands and the relationship of associated factors that might be concerned in its occurrence. Interest concerning the significance of the lesion had been aroused by observing its presence in several thyroid glands removed surgically, while it had been observed rarely in other organs and tissues.

The lesion, although varying in degree in different cases, was of fairly constant morphology and distinct from ordinary arteriosclerosis. In all instances it was manifest only on microscopic study. The least

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From the Department of Pathology, Medical College of Virginia.

1. Curtis, in discussion on Phemister and Delaney: *Arch. Path.* **9**:957, 1930.
2. Curtis and Delaney: *Arch. Path.* **10**:580, 1930.
3. Gutknecht: *Virchows Arch. f. path. Anat.* **99**:419, 1885.
4. Jores: *Beitr. z. path. Anat. u. z. allg. Path.* **21**:211, 1897.
5. Isenschmid: *Frankfurt. Ztschr. f. Path.* **5**:205, 1910.
6. Hesselberg: *Frankfurt. Ztschr. f. Path.* **5**:322, 1910.
7. Wangensteen: *Surg., Gynec. & Obst.* **48**:613, 1929.
8. Karsner: *Human Pathology*, Philadelphia, J. B. Lippincott Company, 1931, p. 853.



conspicuous and presumably earliest lesions were confined to hyalinization or calcification of portions of internal elastic membrane, with fragmentation; these were sufficiently insignificant to be overlooked readily. With increase in calcareous deposition, the convoluted although fragmented contour of the elastica became irregular in thickness or assumed a beaded appearance. In more advanced cases, with extension of the deposits into the media and into the now somewhat thickened intima, the condition was indistinguishable from Mönckeberg's<sup>9</sup> medial calcification of peripheral arteries. In the earlier lesions, degenerative changes in the media immediately adjacent to the calcified portions of the elastic membrane were manifest in vacuolation and fibrillar lysis. Intimal thickening, seldom conspicuous and without plaque formation, limited to fibrillar proliferation, always had the aspect of being secondary. The media in some instances was slightly or moderately hypertrophied.

#### MATERIAL AND PROCEDURE

The material on which the bulk of this study is based consisted of the cases of goiter represented by 100 consecutive partial thyroidec-tomies. Multiple sections of each specimen were prepared, selection of blocks being made largely in reference to visible capsular blood vessels. The average number of sections per case was 5; the extremes were 2 and 22. Sections in selected cases were treated with 1 per cent silver nitrate—von Kossa's method for demonstrating calcium. In studying the sections, particular attention was given to arteries, any deviation from normal being recorded. The condition of veins also was noted, and the presence and character of other lesions of the thyroid gland were reported. In parallel columns, the patient's age, blood pressure, duration of symptoms, functional thyroid state and use of iodine therapy, and the presence of any concomitant chronic disease other than goiter, were tabulated.

Twenty-five nongoitrous and essentially normal thyroid glands of children and adults, derived from routine autopsies, and 50 thyroid glands of fetuses and new-born infants were studied similarly with a view to comparison of the occurrence of the lesion in goiter.

#### OBSERVATIONS

The observations are summarized in tables 1 and 2. Calcification and fragmentation of the arterial internal elastic membrane occurred in 56 of the 100 cases. The morphology and variations of the lesion

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9. MacCallum, W. G.: *A Text-Book of Pathology*, Philadelphia, W. B. Saunders Company, 1931, p. 326.

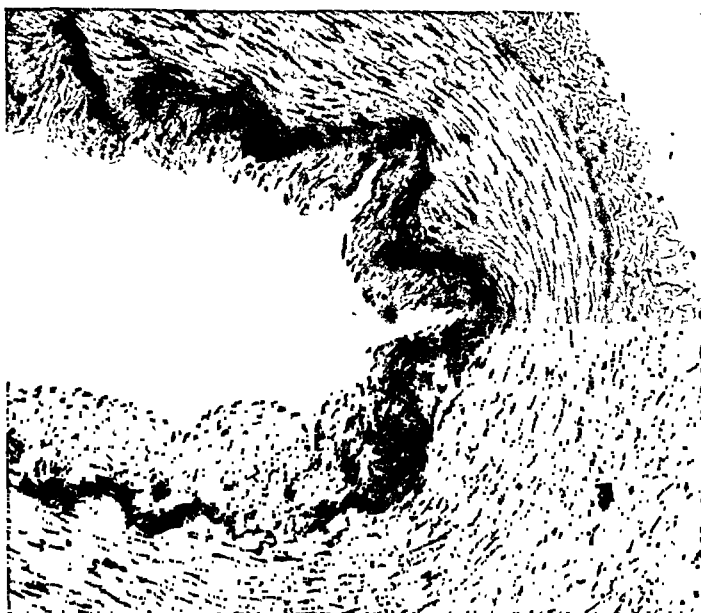


Fig. 1.—A relatively early stage of the lesion. Portions of the internal elastic membrane are calcified. There are moderate degrees of intimal proliferation and medial hypertrophy.

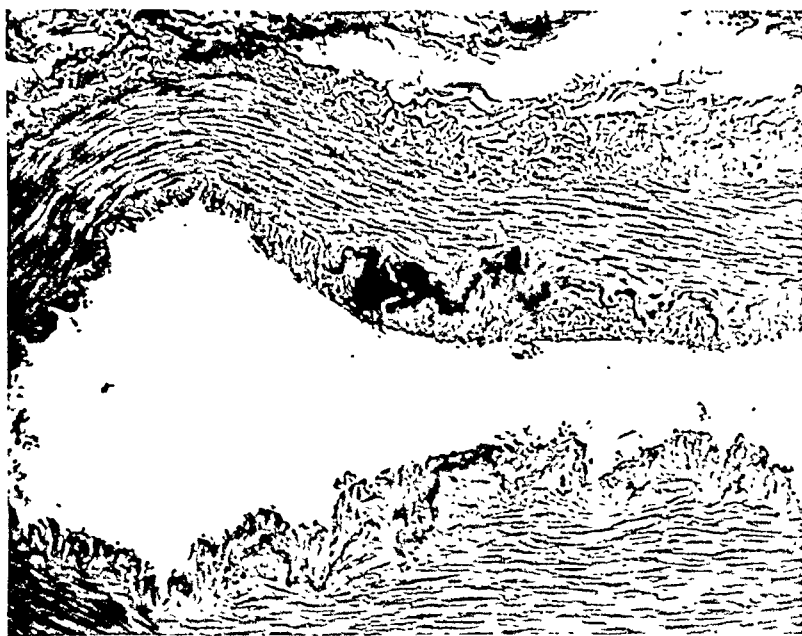


Fig. 2.—An artery of an otherwise normal thyroid gland. A threadlike calcareous band occupies much of the internal elastic membrane, with more bulky calcareous deposition in some portions. Intimal proliferation, focal medial degeneration and medial hypertrophy are associated.

coincide with the description given in an earlier paragraph. In somewhat less than half the cases, the lesion was insignificant, often confined to a threadlike line of calcification along a portion of the internal elastic membrane. In over half of the instances the lesion was prominent, and in a few cases was seen as bulky, calcareous masses occupying most of the thickness of the arterial wall and effecting a structure identical with Mönckeberg's type of medial calcification. Intermediate degrees of involvement filled gaps in a gradual transition from the earliest to the most advanced lesions. Perhaps the development of Mönckeberg's sclerosis is traced here. Limited fibrillar connective tissue proliferation in the intima and varying degrees of medial degen-

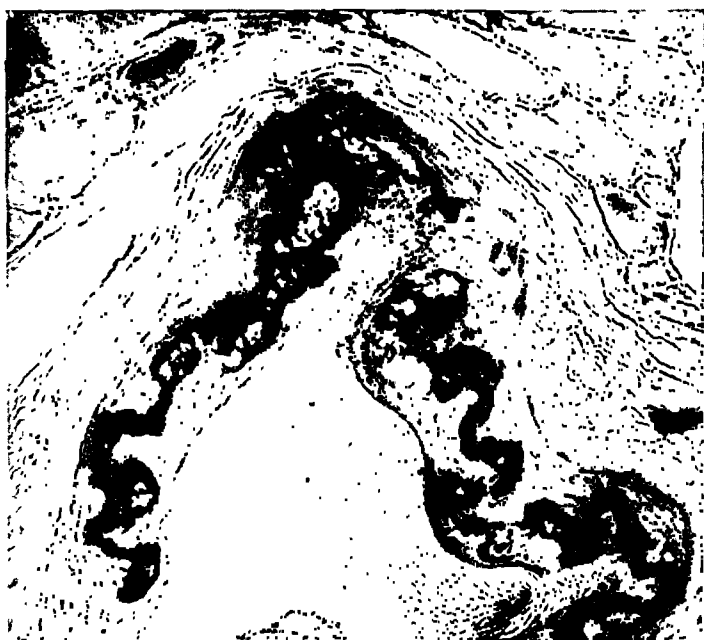


Fig. 3.—An advanced stage, compatible with Mönckeberg's medial calcification, occurring in a diffuse nonhyperplastic goiter.

eration or hypertrophy were invariably associated. By far most of the examples of the lesion occurred in arteries of good size lying in the capsule of the gland.

If the 100 goiters are divided into 5 classes corresponding to morphologic types, and each class is subdivided into two groups—those which include the lesion and those which do not (table 1)—it is found that the lesion has no definite predilection for any type of goiter; the incidence is distributed fairly proportionately over the different classes. Diffuse nonhyperplastic goiter, nodular hyperplastic goiter and neoplasms of the thyroid gland are represented by numbers of cases too small for reliable deductions. Former accounts of the occurrence of

TABLE 1.—Observations in 100 Goitrous Thyroid Glands in Relation to Calcification of the Arterial Internal Elastic Coat

Type of Goiter	Group	Cases	Percentage	Average Age of Patient, Yr.	Average Duration of Goiter, Yr.	Average Blood Pressure	Cases with Hyperthyroidism	Cases with Iodine Therapy	Cases with Concomitant Chronic Disease	Other Lesions of Thyroid Gland
Diffuse Nonhyperplastic	Lesion present	6	75	34.0	3.2	130 — 72	1	3	3: chr. inflam. foci, dent. caries	Nothing irrelevant to type of goiter
	Lesion absent	2	25	23.0	0.7	120 — 75	1	2	2: chr. inflam. foci	Nothing irrelevant to type of goiter
Nodular Nonhyperplastic	Lesion present	14	50	40.0	5.2	137 — 86	2	5	7: chr. inflam. foci, ut. fibroids, cardiac hypertr.	Nothing irrelevant to type of goiter
	Lesion absent	14	50	40.0	10.6	136 — 77	3	5	8: chr. inflam. processes, ut. fibroids	Nothing irrelevant to type of goiter
Diffuse Hyperplastic	Lesion present	25	57	34.6	1.8	132 — 71	25	25	13: chr. inflam. foci, gastric ulc. tb., syph., rheum.	Marked chr. thyroiditis, 2 instances
	Lesion absent	19	43	31.6	1.4	137 — 72	19	19	12: chr. inflam., cardiovasc. dis., ut. malposition	Marked chr. thyroiditis, 1 instance
Nodular Hyperplastic	Lesion present	4	44	37.0	5.6	159 — 86	4	3	2: chr. inflam. processes	Nothing irrelevant to type of goiter
	Lesion absent	5	56	39.0	9.8	161 — 89	5	4	3: chr. inflam. processes	Nothing irrelevant to type of goiter
Neoplasm of Thyroid	Lesion present	7	64	37.4	4.2	152 — 95	0	0	3: pelvic inflam. dis., toxem. of preg., dent. caries	Fibrosis, atrophy, inflam. resp.
	Lesion absent	4	36	33.2	5.0	137 — 73	0	0	3: diabetes, chr. inflam. foci, syphilis	Fibrosis, atrophy, inflam. resp.

TABLE 2.—Occurrence of Calcification of Internal Elastic Membrane in Goitrous and in Nongoitrous Thyroid Glands

	Cases	Number with Arterial Lesion	Percent. with Arterial Lesion
Goitrous thyroid glands.....	100	56	56
Nongoitrous thyroid glands from routine autopsies, children and adults .....	25	3	12
Thyroid glands of fetuses and new-born infants.....	50	0	0

arteriosclerosis in goiter have emphasized its being found in nodular (adenomatous) goiter. In the present study, this group contributed the lowest percentage of occurrence. The least frequent occurrence of the lesion was found in nodular hyperplastic goiter, the lesion being present in 4 cases out of 9 (44 per cent), while the most frequent occurrence was found in the type represented by the smallest number of cases, i. e., diffuse nonhyperplastic (colloid) goiter—the incidence here being 6 out of 8, or 75 per cent. Half of the cases of nodular nonhyperplastic (adenomatous) goiter, 25 of 44 cases of diffuse hyperplastic (exophthalmic) goiter, and 7 of 11 neoplasms of the thyroid gland presented one or more instances of the lesion.

The ages of patients presenting the lesion and of those not presenting it were too close for any deductions in this regard. It was seen to occur at an age younger than that at which appreciable sclerosis is to be expected. The youngest patient in whom it occurred was a young colored woman, 20 years of age, who had diffuse hyperplastic goiter.

The average blood pressure of those having the lesion was about the same as that of those not having it.

The lesion bore no constant relationship to the duration of goiter, to the presence of hyperthyroidism or to iodine therapy. Concomitant chronic disease other than goiter was as frequent and as varied in nature in those having the lesion as in those not having it. The structure of the thyroid gland other than the arterial lesions presented no appreciable differences in the two groups.

In 3 of 25 nongoitrous, essentially normal thyroid glands removed at autopsy, the calcareous arterial lesion was found, and was morphologically identical with the lesion in goiter. The lesion was not observed in sections of arteries of other viscera in these cases. One of these cases was that of a white man, 54 years of age, dying from carbon monoxide poisoning; another, that of a colored man, 37 years old, with intestinal obstruction, and the third, that of a 62 year old colored man, who had a ruptured syphilitic aneurysm.

I believe the occurrence of calcification of the arterial internal elastic membrane in the normal thyroid gland in adults to be higher than this 12 per cent would indicate. The relatively small series included several infants and children, and the average number of sections studied in each case is smaller than that in the goiter series, owing largely to lack of available material.

Hesselberg's observation of arteriosclerosis in the thyroid gland of a new-born infant carries the suggestion that possibly the arterial lesion is congenital. I have studied sections of the thyroid glands of 50

fetuses and new-born infants without finding the lesion; a normal vascular structure was constant throughout.

#### SUMMARY AND COMMENT

An arterial lesion of the thyroid gland, characterized by fragmentation, hyalinization and calcification of the internal elastic lamella, is described. In more advanced cases it conforms with Mönckeberg's medial calcification of peripheral arteries. It occurred in 56 of 100 goiters, irrespective of the age of the patient, blood pressures, duration of the goiter, hyperthyroidism, iodine therapy, presence of concomitant disease or structure of the thyroid gland. It is found also in the normal thyroid gland, but probably less frequently. It was not found in a series of 50 thyroid glands of fetuses and new-born infants. It is rare in tissues other than the thyroid. The significance of the lesion is not manifest; morphologically, it is degenerative.

# PATHOLOGY OF SHOCK

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AND

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PHILADELPHIA

Throughout this discussion the term shock will be used to designate the condition following severe physical injury in which low blood pressure, rapid, weak pulse, decreased blood volume, increased concentration of blood and usually rapid respiration and decreased temperature are prominent features. It will be used as synonymous with the terms circulatory shock, surgical shock, wound shock, traumatic shock or secondary shock, referring to the condition that develops some hours following injury as distinct from that occurring immediately on receipt of injury.

Space does not permit a review of the subject. Adequate reviews are available.<sup>1</sup> A widely accepted view is that shock results from traumatic toxemia; that effective blood volume is reduced by stagnation of blood in dilated peripheral capillaries and venules, and that such withdrawal of blood from currency results in embarrassment or failure of the circulatory function.

Recent experiments have been interpreted to indicate that traumatic toxemia is not a factor, but that shock results from hemorrhage and loss of fluid into the area of trauma. The experiments of Blalock and his associates<sup>2</sup> included intricate combinations of resection of one or both femurs, the application of tourniquets with and without obstruction to one or both femoral vessels, and trauma accompanied by extensive hemorrhages. These were made under barbital anesthesia, which alone fre-

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The work was carried out under the auspices of the Martin Research Foundation.

1. Cannon, W. B.: *Traumatic Shock*, Philadelphia, D. Appleton and Company, 1923. Krogh, A.: *The Anatomy and Physiology of Capillaries*, ed. 2, New Haven, Conn., Yale University Press, 1929.

2. Blalock, A.: *Arch. Surg.* **15**:762, 1927; **20**:959, 1930; **22**:314, 598 and 610, 1931. Blalock, A., and Bradburn, H. B.: *Arch. Surg.* **19**:725, 1929; **20**:26, 1930. Johnson, G. S., and Blalock, A.: *Arch. Surg.* **22**:626 and 855, 1931. Blalock, A.; Beard, J. W., and Johnson, G. S.: *J. A. M. A.* **97**:1794, 1931. Beard, J. W., and Blalock, A.: *Arch. Surg.* **22**:617, 1931. Butler, Virginia; Beard, J. W., and Blalock, A.: *Arch. Surg.* **23**:848, 1931. Harris, P. N., and Blalock, A.: *Arch. Surg.* **22**:638, 1931.

quently produces shocklike phenomena.<sup>3</sup> Blalock recorded that in four of five normal dogs there was from 56 to 110 mm. fall in blood pressure from barbital alone. One normal dog died five hours after the administration of barbital.<sup>4</sup> The viscera showed congestion, edema and capillary hemorrhages such as Krogh<sup>5</sup> describes as the characteristic effects of capillary poisons. In a maze of experimental intricacies, using barbital anesthesia and variation of blood pressure as the criterion of shock, the authors lost all distinction between shock and hemorrhage. They concluded that the cause of low blood pressure after various types of injury was the loss of whole blood or of plasma at the site of the trauma.

Parsons and Phemister<sup>6</sup> drew similar conclusions from experiments in which trauma, under barbital anesthesia, produced hemorrhages of from 300 to 650 cc. The experiments were not designed to distinguish between shock and hemorrhage.

Robinson and Parsons<sup>7</sup> compared the water content of the blood and muscles of dogs. Following hemorrhage, the muscles lost and the blood gained in water content. Following administration of histamine, the muscles gained and the blood lost in water content. Following mild trauma under barbital anesthesia there was a transfer of water from the blood to the muscles as in dogs treated with histamine. These dogs were then subjected to repeated trauma accompanied by hemorrhage, after which a transfer of water from the tissues to the blood occurred. They concluded that loss of blood into traumatized areas causes shock. The conclusion appears contrary to the evidence obtained from the mildly traumatized animals before hemorrhage was made a factor. To produce further trauma and hemorrhage in animals already suffering from shock only introduced confusion.

The opinion that shock is due to hemorrhage might best be tested by comparing the effects of trauma without hemorrhage with those of hemorrhage alone. In each of the experiments mentioned, the shocklike effects of barbital apparently were not considered.

#### TISSUE CHANGES IN EXPERIMENTAL SHOCK

Valuable evidence in the question of trauma vs. hemorrhage as factors in shock may be derived from the appearances of the tissues in those conditions. The ischemia of tissues in death from hemorrhage

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3. Peterson, Frederick; Haines, W. S., and Webster, R. W.: *Legal Medicine and Toxicology* by many Specialists, ed. 2, Philadelphia, W. B. Saunders Company, 1923, vol. 2, p. 661.

4. Blalock, A.: *Arch. Surg.* **22**:604, 1931.

5. Krogh, A.: *The Anatomy and Physiology of Capillaries*, ed. 2, New Haven, Conn., Yale University Press, 1929, p. 195.

6. Parsons, Eloise, and Phemister, D. B.: *Surg., Gynec. & Obst.* **51**:196, 1930.

7. Robinson, William, and Parsons, Eloise: *Arch. Path.* **12**:869, 1931.



is well recognized. The opposite changes have been noted in shock from trauma in human beings and in experimental shock. Morrison and Hooker,<sup>8</sup> Gasser, Erlanger and Meek,<sup>9</sup> Keith,<sup>10</sup> Bayliss,<sup>11</sup> Cannon, Fraser and Hooper,<sup>12</sup> Whipple, Smith and Belt<sup>13</sup> and others have recorded observations on congestion, capillary hemorrhages and edema in shock. Dale, Laidlaw and Richards<sup>14</sup> found exactly these changes following administration of histamine. Krogh<sup>5</sup> found such effects resulting from many capillary poisons. Apparently sufficient emphasis has not been given to these visible changes.

In the experiments reported in this paper, shock was produced in dogs, primarily to secure tissues for study. Incidentally, these experiments have some bearing on the etiology. In shock the blood becomes concentrated. It is common knowledge that dilution of blood occurs following hemorrhage. The averages of the red cell count, hemoglobin percentage and specific gravity of the blood for a period of from five to ten days prior to the experiments were compared with variations noted during and following the experiments. When records of the blood pressure were made, morphine, 0.2 mg. per kilogram of body weight, was given hypodermically. Then, under local procaine hydrochloride anesthesia, an arterial cannula was inserted and connected with a mercury manometer. No prolonged continuous records were attempted.

Procedures were devised to produce shock without local injury and hemorrhage. A normal dog was killed under ether anesthesia. Quantities of muscle were excised immediately and ground through a meat chopper. This, diluted with an equal quantity of physiologic solution of sodium chloride, was sterilized by autoclave and refrigerated until used.

Freshly ground, unsterilized muscle was extracted with an equal volume of distilled water. The fluid was squeezed out through gauze and centrifugated at high speed for fifteen minutes. The layer of fat from the top and the sediment from the bottom were discarded. Sodium

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8. Morrison, R. A., and Hooker, D. R.: *Am. J. Physiol.* **37**:86, 1915.

9. Gasser, H. S.; Erlanger, J., and Meek, W. J.: *Am. J. Physiol.* **49**:90 and 151, 1919; **50**:31, 1919.

10. Keith, N. M.: Medical Research Committee, Special Report Series, no. 26, 1919, p. 36.

11. Bayliss, W. M.: Medical Research Committee, Special Report Series, no. 25, 1919, p. 26; no. 26, 1919, p. 24.

12. Cannon, W. B.; Fraser, John, and Hooper, A. N.: Medical Research Committee, Special Report Series, no. 25, 1919, p. 72; *J. A. M. A.* **70**:526, 1918.

13. Whipple, G. H.; Smith, H. P., and Belt, A. E.: *Am. J. Physiol.* **52**:72, 1920.

14. Dale, H. H.; Laidlaw, P. P., and Richards, A. N.: Medical Research Committee, Special Report Series, no. 26, 1919, p. 8; *Brit. M. J.* **1**:381, 1917; *J. Physiol.* **52**:110, 1918; 335, 1919.

chloride was added to render the fluid isotonic. It was passed through paper filters, sterilized by Berkefeld filtration, and stored in refrigeration. A watery extract of traumatized muscle was similarly prepared from a dog in which shock had been produced by bruising.

*Dog S-24.*—Under surgical asepsis, 200 Gm. of sterilized normal muscle was introduced into the peritoneal cavity of an adult male mongrel weighing 6.32 Kg. The operation was done under ether anesthesia lasting twenty minutes. The dog recovered well from the operation. He was active and even playful. Presently he refused all food, but drank water freely. He appeared acutely ill, refused to move about and shivered frequently. There were frequent bowel movements, and the feces contained blood-tinged mucus. The extremities became cold to the touch; respirations and pulse were increased to twice their normal rates. He died ten and one-half hours following the operation. Changes in the concentration of the blood were as shown in table 1.

After death there was about 150 cc. of thin, blood-tinged fluid in the peritoneal cavity. The peritoneum was intensely congested, and the blood vessels along the

TABLE 1.—*Changes in Concentration of Blood of Dog S-24*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	
(Average normal).....		1.059	98	5,820,000	
3/24/32	6:30 a.m.	1.060	101	5,110,000	
	7:00 a.m.	Operation			
	10:30 a.m.	1.065	94	4,860,000	
	1:30 p.m.	1.065	104	5,694,000	
	3:20 p.m.	1.067	108	5,695,000	
	4:50 p.m.	1.075	120	6,400,000	
	5:15 p.m.	1.075	130+	7,450,000	Death

mesenteric attachment were markedly distended. The bowels contained bloody mucus. Congestion was marked in the mucosa of the bowels and in the liver, kidneys, spleen, lungs and myocardium. Petechial hemorrhages were present in the lungs and in serous surfaces.

Microscopic examination showed numerous hemorrhages associated with marked congestion of the capillaries and venules in each of the organs mentioned (fig. 1).

Four other dogs similarly treated showed features identical with those described. The data concerning these are summarized in table 2. Evidently some substance capable of producing rapidly a fatal shocklike condition is absorbed from sterile muscle implanted in the peritoneal cavity. There was no loss of blood by hemorrhage in these experiments.

Sterile aqueous extract of normal muscle produced similar effects in dog S-11 when introduced intraperitoneally. It produced an immediate fall in blood pressure when introduced intravenously in the following experiments:

*Dog S-8.*—An adult male mongrel, weighing 21.3 Kg., was connected with a mercury manometer. An intravenous injection of 20 cc. of watery extract of

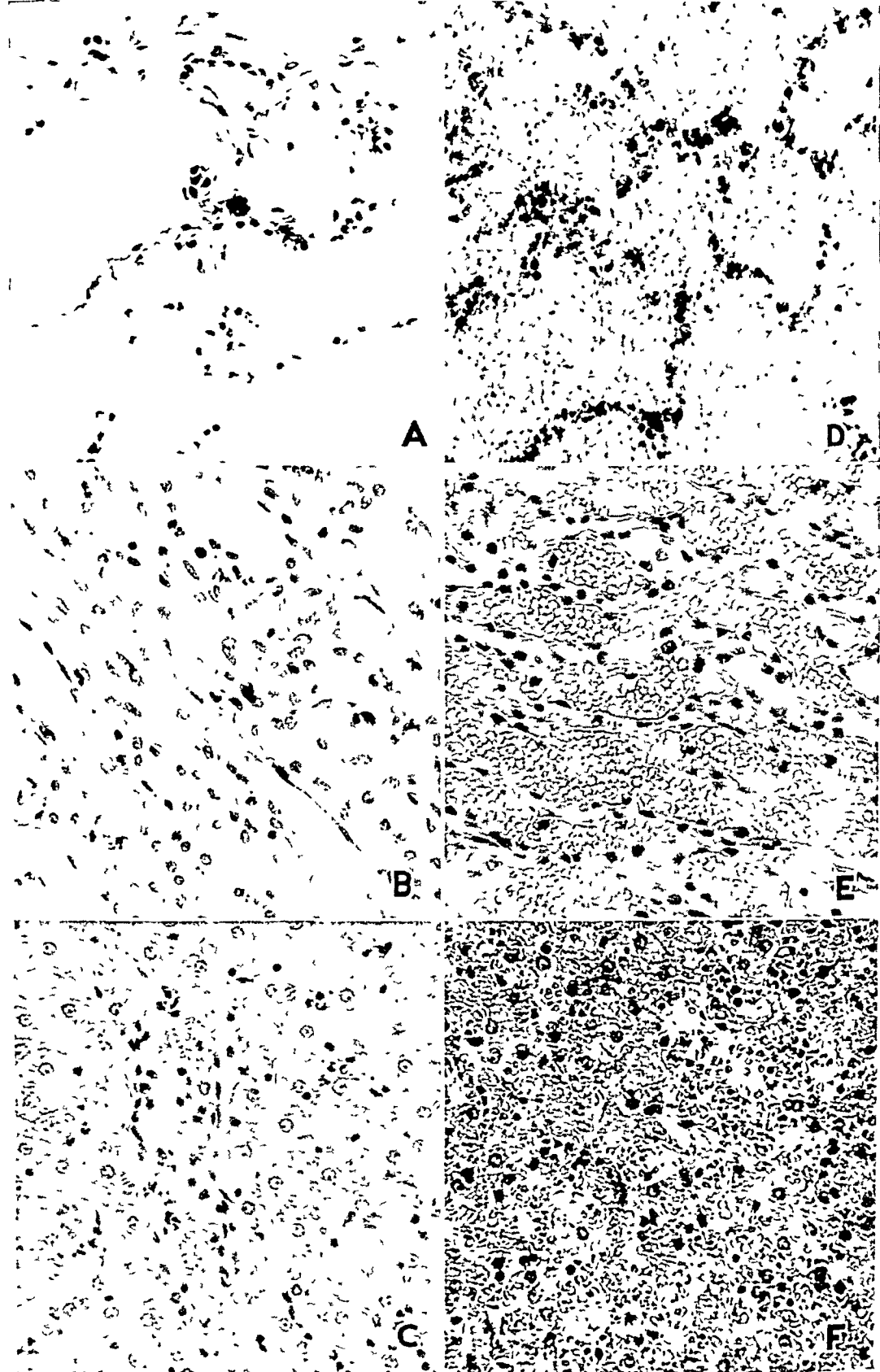


Fig. 1.—*A, B* and *C* are photomicrographs of lung, kidney and liver following death produced by repeated hemorrhages within forty-eight hours. *D, E* and *F* are photomicrographs of lung, kidney and liver following death in fifty-two hours after trauma to muscles.

normal muscle caused the blood pressure to fall 110 mm. During the following two hours the pressure gradually rose 64 mm. The animal recovered.

*Dog S-9.*—The sterile filtrate from 25 Gm. of normal muscle was incubated for twenty hours to permit action of such ferments as it might contain. In a dog weighing 10.25 Kg., intravenous injection of 10 cc. of the filtrate caused the blood pressure to decline 56 mm. The remainder of the filtrate was given in three injections at intervals during an hour. This caused the blood pressure, 140 mm. before the experiment, to drop to 44 mm.—a fall of 96 mm. During eighteen hours the pressure rose to 110 mm. and recovery might have followed. Since it was desired to observe tissue changes, the dog was killed painlessly. The lungs were congested and mottled with numerous dark red areas, which microscopic examination showed to be due to extravasation of blood from capillaries. There was moderate edema of the lungs. The kidneys were markedly congested; otherwise the organs appeared normal.

*Dog S-14.*—A sterile filtrate of traumatized muscle was injected intravenously into a male mongrel weighing 7.28 Kg. Seven injections of 25 cc. each were made at intervals during a period of two hours. This produced marked disturbances of the pulse, respiration and general condition. Fifteen hours later the animal did not react to stimuli and was very ill, with respirations 40 per minute, pulse imperceptible and extremities cold. The hemoglobin had risen from 80 to 105 per cent, and the erythrocytes from 5,400,000 to 6,840,000—about 25 per cent increased concentration of the blood. There were frequent evacuations of blood-tinged mucus from the bowels. The animal died in twenty-one hours.

The gross and microscopic changes were identical in character with those of dog S-24. The congestive features were most marked, and there was widespread central necrosis in the hepatic lobules.

For other similar experiments, see table 2. The effect of muscle extract on these dogs closely resembled that which may follow administration of histamine.

The following experiment illustrates the effects of hemorrhage on the composition of the blood and on the tissues of the body.

*Dog S-23.*—The dog was a female mongrel weighing 10.46 Kg. For six days prior to the experiment, the hemoglobin percentage, red cell count and specific gravity of the blood were determined (table 3).

It will be seen that a loss of 700 cc. of blood within thirty-six hours was sustained by a dog of small size. Assuming the blood to have an average specific gravity of 1.050, this amounted to 7 per cent of the body weight or about 70 per cent of the total blood volume. Such a loss of blood reduced the hemoglobin only to 36 per cent and the specific gravity to 1.032. At this time, the dog showed no evidence of illness. It was active and playful as normally, and undoubtedly would have recovered. It required a further loss of 400 cc. of blood to cause death.

After death there was marked anemia of all the organs and tissues. The lungs were very pale throughout and showed neither congestion nor areas of hemorrhage (fig. 2). The heart muscle was pale brown. There were no petechiae in the endocardium or elsewhere. The spleen, liver and kidneys were dry on section and showed no congestion. All the peritoneal surfaces were pearly white. The venules along the mesenteric attachment were not visible. The mucosa of the entire gastro-intestinal tract was dull white. On microscopic examination, all the tissues had a normal appearance except that there was less blood present than normally (fig. 1).

TABLE 2.—*Summary of Experiments Not Described in the Text*

Dog	Treatment	Change in the Blood	Result	Capillary Congestion	Edema	Capillary Hemorrhage
S-7	Filtrate of traumatized muscle, 175 cc., intravenously injected	No data	Died in 16 hr.	Lungs, bowel, cardiac muscle, kidney, liver	Lungs	Lungs, kidney, liver
S-11	Filtrate of normal muscle, 275 cc., intraperitoneally injected	Increased concentration	Killed in 24 hr.	Peritoneum, intestinal mucosa, liver, lungs	Peritoneal cavity, submucosa	Endocardium, mucosa of bowel, renal cortex
S-13	Filtrate of normal muscle, 235 cc., intravenously injected	Increased concentration	Died in 23 hr.	Peritoneum, intestinal mucosa, spleen	Intestinal mucosa	Endocardium, spleen
S-3	Normal muscle fresh, 40 Gm., intraperitoneally injected	No data	Died in 16 hr.	Lungs, spleen, lymph node, kidney, liver, peritoneum, myocardium, gastro-intestinal tract	Peritoneum	Lungs, kidneys
S-18	Sterile muscle substance, 350 Gm., intraperitoneally injected	No data	Died in 17 hr.	Lungs, liver, kidney, peritoneum, gastro-intestinal tract	Peritoneum, liver, skeletal muscle	Endocardium, lungs, kidney, gastro-intestinal mucosa
S-21	Sterile muscle, 200 Gm., in peritoneum	Increased concentration	Died in 12 hr.	Lungs, liver, spleen, myocardium, kidney	Lungs, skeletal muscle	Lungs, liver, kidney
S-22	Sterile muscle, 100 Gm., in peritoneum	Increased concentration	Killed in 32 hr.	Lungs, liver, myocardium, kidney	Skeletal muscle	Lungs, kidney
S-6	Trauma to muscles of thigh	Decreased concentration, 11 per cent	Died in 32 hr.	Lungs, gastro-intestinal tract, myocardium, liver, kidney	Lungs, submucosa of bowel	Pleura, endocardium, respiratory mucosa, lungs
S-12	Trauma to muscles of thigh	Increased concentration	Died in 22 hr.	Lungs, liver, peritoneum, gastro-intestinal tract, myocardium	Lungs, respiratory mucosa, mediastinum	Endocardium, lungs, kidneys, mucosa of bowel

\* The same gross and microscopic changes were present as described for dog S-24.

TABLE 3.—*Changes in Concentration of Blood Following Hemorrhage*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	Blood Withdrawn, Cc.
(Average normal).....		1.056	92	5,200,000	...
3/15/32	11:00 a.m.	1.055	98	5,400,000	100
	3:00 p.m.	1.055	97	6,320,000	100
	5:00 p.m.	1.055	95	5,300,000	...
3/16/32	10:00 a.m.	1.055	80	5,400,000	100
	11:30 a.m.	1.050	75	4,910,000	100
	1:00 p.m.	1.047	57	4,400,000	100
	2:30 p.m.	1.042	60	3,890,000	100
	4:15 p.m.	1.037	49	3,420,000	100
3/17/32	9:00 a.m.	1.032	36	1,380,000	...
	9:30 a.m.	.....	..	.....	400    Death

We wish to emphasize the contrast between the findings in this animal and those that accompany the shock syndrome.

The effects of barbital are shown by the following experiment:

*Dog S-19.*—A vigorous adult male, weighing 13.6 Kg., was given 4 Gm of sodium barbital intravenously. This is the dosage, 0.3 Gm. per kilogram, used by several authors of articles on shock. Three hours later, the blood pressure had declined 94 mm., and the pulse and respirations were twice their former rates. Beginning at this time, 100 cc. quantities of blood were withdrawn at intervals of thirty minutes. The dog died following the loss of 500 cc. of blood—less than 4 per cent of the body weight.

The lungs were mottled with areas of congestion. There were numerous petechial hemorrhages in the endocardium. There was congestion of the liver, spleen, kidneys and gastro-intestinal mucosa, notwithstanding the fact that death was caused by hemorrhage.

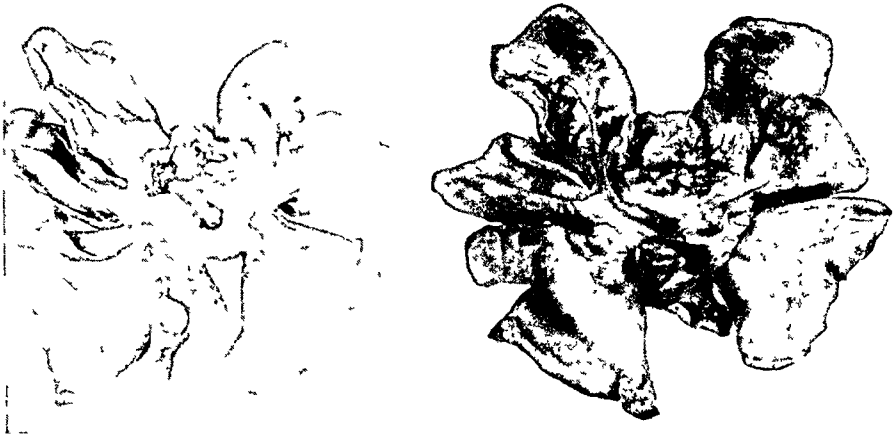


Fig. 2.—Lungs of dogs S-23 (death from repeated hemorrhages within forty-eight hours) and S-24 (shock from muscle substance in the peritoneal cavity).

Barbital alone causes marked fall in blood pressure, increased pulse and respiratory rates, and inability to recover from hemorrhage. The tissue changes and physiologic effects of barbital resemble those of shock.

In the next experiment, shock was produced by trauma:

*Dog S-25.*—At 1:15 p. m., an adult mongrel male, weighing 10.47 Kg., was anesthetized with ether, and the muscles of the hindlegs were severely bruised. As the dog returned to consciousness, pain was prevented by hypodermic injections of morphine, 0.5 mg. per kilogram of body weight at intervals. The dog refused food, but drank water. On the following day, he appeared normal except for marked swelling of the thighs. He was given ether, and the muscles were retraumatized moderately. On March 24, his condition was about the same as on the 23rd. Following heavy massage under light ether anesthesia, a condition of collapse rapidly developed. The pulse and respirations became rapid and the

extremities cold. He became unresponsive to stimuli and died two hours later. The changes in the blood are shown in table 4. Note that at one period there was a decrease in concentration of the blood. This would indicate that hemorrhage was perhaps a factor.

In dogs treated with sterile filtrate of normal muscle, with filtrate of traumatized muscle, with sterile muscle substance implanted in the peritoneal cavity and with mechanical trauma, similar physiologic disturbances resulted. Tissue changes of an identical nature were found at autopsy. These changes included widespread capillary dilatation, especially marked in the lungs, the gastro-intestinal tract, the liver and the kidneys. There were numerous capillary hemorrhages, and edema was present frequently. A prominent antemortem feature was increased concentration of the blood.

TABLE 4.—*Changes in Concentration of Blood of Dog S-25*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	
(Average normal).....		1.058	94	5,840,000	
3/22/32	4:30 p.m.	1.058	101	6,000,000	
3/23/32	10:30 a.m.	1.060	99	5,420,000	
	1:00 p.m.	1.057	101	6,150,000	
	4:30 p.m.	1.055	90	5,900,000	
3/24/32	8:00 a.m.	1.053	86	3,800,000	
	1:30 p.m.	1.054	60	4,100,000	
	4:30 p.m.	1.057	70	4,500,000	
	7:30 p.m.	1.064	96	5,900,000	
	8:00 p.m.	1.065	93	5,990,000	Death

#### OBSERVATIONS IN HUMAN CASES OF SHOCK

In human cases apparently of shock, we have found the same capillary phenomena as in the experiments on dogs.

A white woman, aged 65, was operated on for the removal of an ovarian tumor. Ether was used, and the anesthesia lasted thirty-five minutes. The operation, which was complicated by the presence of adhesions, lasted twenty-five minutes. No significant hemorrhage occurred, and the condition was recorded as fair at the close of the operation. Collapse developed. The pulse became rapid and feeble, the skin pale, the extremities cold and the respirations 30 to 40 per minute. She was given 600 cc. of saline solution intravenously and caffeine and atropine hypodermically. Heat was applied, and further stimulation was attempted with drugs. These were ineffective. The blood pressure fell to 80 mm. systolic and 70 mm. diastolic. She became progressively weaker and died within six hours. There was no evidence of gross hemorrhage. About 2 liters (2,000 cc.) of thin, blood-tinged fluid was present in the peritoneal cavity. The entire intestinal tract was relaxed, atonic and markedly congested. The lungs were dark red and contained a large amount of fluid. The left lung weighed 310 Gm., and the right, 490 Gm. There were concretions in the gallbladder, and the kidneys were arteriosclerotic. No other significant changes were present.

On microscopic examination, the alveoli of the lungs contained variable amounts of serous fluid; some were filled, other partially filled, and others contained air. The capillaries were widely distended with corpuscles. No gross hemorrhages were present, but many corpuscles had escaped into the alveolar spaces. The capillaries of the mucosa of the bowel were distended, and the submucosa was edematous.

Permission was granted by Surgeon General Patterson to study material collected by the medical service of the American Expeditionary Forces in France. Accordingly Major P. E. McNabb, curator of the Army Medical Museum, submitted twenty-six postmortem records selected as those of probable cases of shock. Records of cases of injury to the brain or cord and of cases of penetration of the chest or abdomen were rejected. There remained twenty-two records of multiple lacerated shell-fragment wounds of the arms, legs, shoulders or buttocks. In each of twenty cases several sections of lung were available for study. The cases fell into groups as follows:

Group 1 consisted of eight cases in which no gas bacillus infection occurred, and in which hemorrhage was a minor factor. The following record (accession no. 4259) is representative of this group:

A soldier had suffered extensive lacerated shell wounds of both feet, with comminuted fractures of the feet and legs. He was brought in with a tourniquet about each leg. Heat was applied, and hot fluids were given by mouth. The tourniquets were released. Five hours later the blood pressure was 70 mm. systolic and 58 mm. diastolic. Gum acacia solution 750 cc. given intravenously raised the blood pressure 13 points. His condition did not improve, and he died in shock eight hours after admission, although no hemorrhage had occurred in the interim.

Postmortem examination showed marked posterior lividity, edema of the brain and marked venous engorgement of the viscera. Both lungs were heavy and livid red, and the crepitation was diminished. They contained numerous areas of petechial hemorrhage. Blood and fluid in increased amount flowed from the surfaces made by sectioning. There were petechial hemorrhages in the endocardium. On microscopic examination, the lungs showed marked capillary engorgement, numerous areas of capillary hemorrhage and moderate diffuse edema.

Group 2 embraced five cases of injuries similar to those in group 1, but complicated by varying degrees of gas bacillus infection. In each case, the record showed venous engorgement, marked pulmonary hyperemia, marked edema of the lungs and petechial hemorrhages in the lungs or in serous surfaces. On microscopic examination, the sections of lung showed no difference from those in group 1.

Group 3 was composed of four cases in which moderate acute anemia from hemorrhage was present. Gas infection was present in one of these. In two cases, the lungs had normal consistence and crepitation. Microscopically there were edema, congestion and capillary hemorrhages. Anemia and the shock mechanism appeared to be combined. The other two cases showed no essential differences from those described under group 1. Grossly and microscopically, the lungs showed congestion, edema and capillary hemorrhages in marked degree. Ischemia was not present. The case in which gas infection was present showed no gross or microscopic differences from those of traumatic shock alone.



Group 4 included five cases in which hemorrhage seemed a major factor. Gas infection was present in one of these. The following case (accession no. 4344) is representative of four in this group:

A soldier had experienced a shell fragment injury to the right lumbar region. He was admitted in extreme shock and given the usual treatment for shock. The time of injury and of death were not recorded. At postmortem examination there was very slight posterior lividity. Free blood, 1,500 cc. in amount, was present in the peritoneal cavity. The lungs varied in color from light gray to light pink. The crepitation was normal. The surfaces made by sectioning gave up a smaller amount of fluid than normally. On microscopic examination, the sections of the lungs contained little visible blood, and there was no edema or areas of capillary hemorrhage.

Three other cases showed similar features. The viscera were pale and anemic. "Dry autopsy" was noted in the records. Gross and microscopic examinations of the lungs showed less blood than normally. The examinations indicated that hemorrhage was the chief cause of death. In the fourth case, gas bacillus infection was present. The patient lived three days following injury, and no hemorrhages occurred in the interim. The gross and microscopic features were those of shock.

Summarizing the observations made at the time of autopsy in the seventeen cases in groups 1, 2 and 3 in which shock was a major factor, one finds marked pulmonary congestion and edema in sixteen cases, petechial hemorrhages in serous surfaces in eight cases and visceral venous engorgement in eleven cases.

Microscopic study of sections of the lungs showed marked capillary congestion with varying degrees of capillary hemorrhage and edema in fifteen cases in this series. In two cases no sections were available.

It is noteworthy that the changes present in these cases are not characteristic of anemia from hemorrhage, and that they were absent in the cases of group 4, in which hemorrhage was assigned as the chief cause of death.

#### COMMENT

Most conditions of disease are accompanied by tissue changes which are more or less characteristic, and which both aid in the recognition of the cause and corroborate the correct etiologic interpretation. Shock is not, as has been supposed, an exception to the general rule.

A significant feature of shock is increased concentration of the blood. This has been mentioned frequently in previous reports. That hemorrhage is often a complicating factor of most serious gravity in shock is generally admitted. Trauma of sufficient severity to produce shock in dogs is accompanied by varying degrees of hemorrhage. In one traumatized animal, S-6, a decrease of 11 per cent in the concentration of the blood was observed. The death of this dog may have been due to a combination of shock and hemorrhage. That death was not due to hemorrhage alone was indicated by the marked congestion, edema and numerous capillary hemorrhages in the viscera. Those who have had

difficulty in drawing distinctions might have observed whether the blood and tissues of shocked animals showed the changes that serious hemorrhage will produce.

We have noted that edema, particularly of the lungs, is more marked in cases of shock in man than in experimental shock in animals.

#### CONCLUSIONS

The shock syndrome is accompanied by gross and microscopic changes opposite in character to those produced by hemorrhage. The changes include dilatation and engorgement of capillaries and venules, permeability of capillary walls, as indicated by petechial hemorrhages and edema, and frequently effusion into serous cavities. Increased concentration of the blood is a characteristic phenomenon in shock.

These vascular phenomena are widespread, but are most prominent in the pulmonary and gastro-intestinal tracts.

These circulatory effects can be produced by trauma, by intravenous or intraperitoneal injections of extract of normal muscle or by implanting muscle substance into the peritoneal cavity. This tends to corroborate the view that products absorbed from injured tissues produce the shock syndrome, and that these act by causing dilatation and increased permeability of the capillaries and venules.

Barbital produces physiologic and histologic changes resembling those of shock. Drugs having such properties should not be used when normal blood pressure and capillary tonus are important.

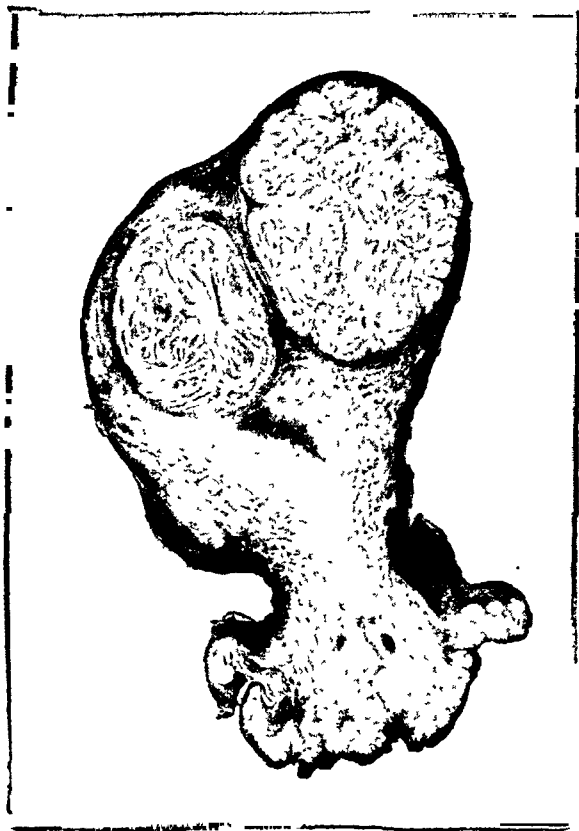
# Laboratory Methods and Technical Notes

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## PRESERVATION OF THIN SECTIONS OF TISSUE IN NATURAL COLORS

JAMES E. DAVIS, M.D., AND ARTHUR L. AMOLSCH, M.D., DETROIT

Satisfactory preservation of valuable specimens has always been a problem. The complete specimen preserved in the large jar is unsatisfactory because of the space required for storage, the inaccessibility for



A thin section of a uterus and cervix with multiple myomas of the uterus and carcinoma of the cervix. The outer black frame is asphalt. The narrow light border is brass. The two faces of the picture are glass. The figure is reduced from 5 by 7 inches (12.7 by 17.78 cm.).

close examination, the liability to deterioration and the difficulty it offers in transportation for class or convention work.

The essential requirements for a permanent gross exhibit of pathologic change in tissue may be fully met by securing a thin section

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through the organ so as to display the largest possible cross-section area of the typical pathologic changes. By preserving this thinly cut section in natural colors in a suitable fluid contained in a thin, light cell, the requirements of small storage space, maximum teaching values, satisfactory transportation, etc., have been satisfactorily met.

This type of cell and this method of preservation have now been in use in our laboratory for more than two years, and have proved to be the most satisfactory method of exhibiting gross tissues known to us. The cells have been transported over long distances and roughly used, and have shown a very desirable permanency.

#### CONSTRUCTION OF CELL FOR THE MOUNTING OF A THIN SECTION OF TISSUE

- Materials Required.*—1. Glass plates 5 by 7 inches (12.7 by 17.78 cm.).  
 2. One-fourth inch strips of 15-gage soft brass,  $16\frac{3}{4}$  inches (42.52 cm.) long.  
 3. Refined Trinidad Lake Asphalt (Genasco Brand, The Barber Asphalt Co., Philadelphia). Do not use commercial pavement asphalt.  
 4. Battleship linoleum,  $7\frac{1}{4}$  by  $4\frac{1}{2}$  by  $\frac{1}{4}$  inches (18.4 by 11.4 by 0.6 cm.).  
 5. Heavy spring paper clamps.  
 6. Steel spatula or putty knife.

The glass plates should be well cleansed and freed from oil by the use of sapolio or a similar substance and hot water.

The brass strips should not vary appreciably in width from end to end and should be filed or ground to an even square-cut edge. They should be cleaned and polished before the construction of the cell is begun. The strips should be cut about one third through at the points of bending, with a chisel or file. When adjusted about the linoleum mould there should be no spring exerted inward or outward.

Discoloration of the brass strips by certain tissues, such as liver, is effectually prevented by dipping the strips in duco or varnish twice, i. e., by giving them two coats of this substance.

The lump asphalt should be heated to melting several times and stirred until it is no longer frothy. Care must be exercised to prevent charring due to overheating.

*Method of Assembling.*—1. The bent brass strip is placed about the linoleum and adjusted to a perfect fit.

2. A plate of glass is placed on each side of the linoleum, and two spring clamps are used to hold them in position. The mould is then centered and the glasses are properly alined. Two short pieces of brass are temporarily inserted above the ends of the brass strips and held against the projecting linoleum by means of rubber bands. This will insure a uniform limitation of the asphalt to the open end of the cell and permit easy insertion of the final closing strip of brass.

3. A hot spatula is dipped into the molten asphalt and carried to the brass strip and smeared onto the glass plate, eliminating any air spaces. This procedure is followed on both glasses along one edge and finally the asphalt is filled into the full length of the groove and permitted to harden. The other two sides are treated in the same manner. It will be necessary to add some additional asphalt at the corners.

Finally the free margin of the asphalt is smoothed with a hot spatula; or the edge of the asphalt may be softened slightly with a Bunsen flame and then the edge may be pressed gently on a smooth board or strip of metal to obtain a smooth, even surface. Excess of asphalt on the glass surfaces may be removed with a knife or a razor-scraper.

4. The linoleum mould is now withdrawn, and the two short brass insertions are removed. The cell is now ready for the tissue to be inserted.

5. Fill the cell with the mounting fluid (50 per cent cane sugar saturated with thymol) to a level about  $1\frac{1}{2}$  inches (3.8 cm.) from the top.

6. Insert a long, thin, flat brain knife between the specimen and the glasses and liberate all imprisoned air. If the fluid becomes turbid during this operation, the turbid fluid may be displaced by inserting a long needle or cannula to the bottom of the cell and by permitting clear sugar solution to flow in by gravity.

7. Pour out excess fluid to  $1\frac{1}{2}$  inches (3.8 cm.) from the top and thoroughly dry the inner surfaces of the upper ends of the glasses and asphalt.

8. Insert the final strip of brass, provided with a hole bored close to one end, so that it rests on the end of the upright strips. Paste a bit of paper over the hole.

9. Fill the upper edge with asphalt, as before described.

10. With a hot needle, punch through the asphalt over the filling hole in the brass and fill the remainder of the cell by means of a hypodermic syringe and needle displacing all air.

11. Dry out the hole in the asphalt and finally sear with a hot knife point to remove all moisture and sugar.

12. Fill the hole with molten asphalt.

13. Clean the cell and finally coat the asphalt with duco to prevent stickiness.

#### PRESERVATION OF TISSUES ADAPTED TO THE CELL

The tissue should be fixed promptly in Pick's solution. The surface blood should be removed, preferably with physiologic solution of sodium chloride to prevent hemolysis.

Anatomic relationships should be maintained by pinning the fresh specimen to yucca board or suspending it on a glass frame. This will prevent undue warpage and distortion.

If a large specimen or an entire organ is to be fixed, such as a liver, lung or spleen, uniform fixation will be better obtained by perfusing Pick's solution through the vascular channels and suspending the organ in a large amount of the fixative.

If possible, avoid cutting the fresh specimen, because unnatural distortion due to contraction will occur, and the blood will be drained from the tissue.

If only a portion of a large organ is desired, it is best to cut a slice about 1 inch (2.5 cm.) thick and permit this to fix without distortion.

Preservation of color and proper fixation of the tissue depend, first, on maintaining the chemical concentration of the solution, and second, on the time of fixation.

All specimens should be placed in at least twice their volume of fluid. Small specimens will be well fixed in from three to five days. Large specimens, such as a spleen, breast, liver or brain, may require from five to fourteen days.

Repeated use of a given volume of fluid will deplete the chemical content and cause dilution, so that good results may not be expected.

After proper fixation, the specimen is thoroughly washed in running tap water to remove all chemicals.

The tissue section is now made, a smooth board being used, provided with two parallel 8 mm. glass rods to serve as a knife guide. A long, thin, flat blade from 14 to 16 inches (36 to 37 cm.) in length is drawn with a sweeping movement through the specimen, which is held on the cutting board between the glass rods.

The specimen is now ready to be mounted in cane sugar solution.

*Formula for Pick's Fixing Solution.*—Pick's solution is prepared as follows:

Chloral hydrate . . . .	50 Gm.	100 Gm.	1,000 Gm.
Carlsbad salts . . . . .	50 Gm.	100 Gm.	1,000 Gm.
Formaldehyde . . . . .	100 cc.	200 cc.	2,000 cc.
Distilled water . . . . .	1,000 cc.	2,000 cc.	20,000 cc.

Carlsbad salts are prepared as follows:

Sodium sulphate . . . . .	22 Gm.	220 Gm.
Sodium bicarbonate . . . . .	20 Gm.	200 Gm.
Sodium chloride . . . . .	18 Gm.	180 Gm.
Potassium nitrate . . . . .	38 Gm.	380 Gm.
Potassium sulphate . . . . .	2 Gm.	20 Gm.
Total . . . . .	100 Gm.	1,000 Gm.

*Jore's Solution.*—Soft tissues, such as brain or myxomatous tumors, may be fixed in Jore's modification of Pick's solution. This has less chemical concentration and lower formaldehyde content, so that fixation is accomplished more slowly. It is prepared as follows:

Carlsbad salts . . . . .	125 Gm.	625 Gm.
Chloral hydrate . . . . .	125 Gm.	625 Gm.
Formaldehyde . . . . .	125 cc.	625 cc.
Distilled water . . . . .	4,000 cc.	20,000 cc.

Carlsbad salts (for 20,000 cc. of distilled water) should be made up as follows:

Sodium sulphate . . . . .	137 Gm.
Sodium bicarbonate . . . . .	125 Gm.
Sodium chloride . . . . .	113 Gm.
Potassium nitrate . . . . .	233 Gm.
Potassium sulphate . . . . .	17 Gm.
Total . . . . .	625 Gm.

*Mounting Fluid.*—Fifty per cent cane sugar in distilled water is heated to the boiling point; sufficient thymol crystals are added to saturate; the fluid is filtered through paper or porcelain, and kept well stoppered.

Do not add acid, alkaline or metallic substances to the sugar solution as a preservative, since they attack the brass. Formaldehyde should *not* be used.

## A MODERN AUTOPSY TABLE

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The usual commercial autopsy table is of white porcelain, which is difficult to keep clean; it has a central drain on which the body must rest, so that, as the autopsy proceeds, there is an ever growing pool of blood and fluid around the body. H. E. Robertson<sup>1</sup> has devised a table consisting of a large, shallow pan, with a central drain, and a top of slats or cross-pieces which hold the body up above the pan. Through the generosity of Dr. Irving Potter and Dr. Milton Potter, a new autopsy table was recently made available for the Millard Fillmore Hospital in Buffalo. This table (figure) was constructed by the Jeffrey-Fell Company of that city, on the same principles as the one described by H. E. Robertson, but with certain improvements.

The frame of the table is of galvanized iron supported on legs of galvanized iron pipe, and this has the advantage of both strength and lightness. The height of the table can be easily changed to fit the heights of succeeding pathologists by disconnecting the lowest joint in each leg and fitting in a longer or shorter length of pipe. The frame around the top is covered by monel metal, to which is attached a shallow pan of monel metal sloping gently from the edges to a central drain which is 2 inches in diameter and 2 inches lower than the edges of the pan. This drain communicates directly with a 6 inch (15.24 cm.) sewer in the hospital and is covered by a convex cap of monel metal perforated with openings about  $\frac{1}{4}$  inch (0.61 cm.) in diameter, which allows the free passage of fluids into the sewer, but holds back all tissue of appreciable size. At one side of this cap is a small curved handle by which it can be quickly and easily removed from the drain when larger pieces of tissue or blood clots are to be washed down into it.

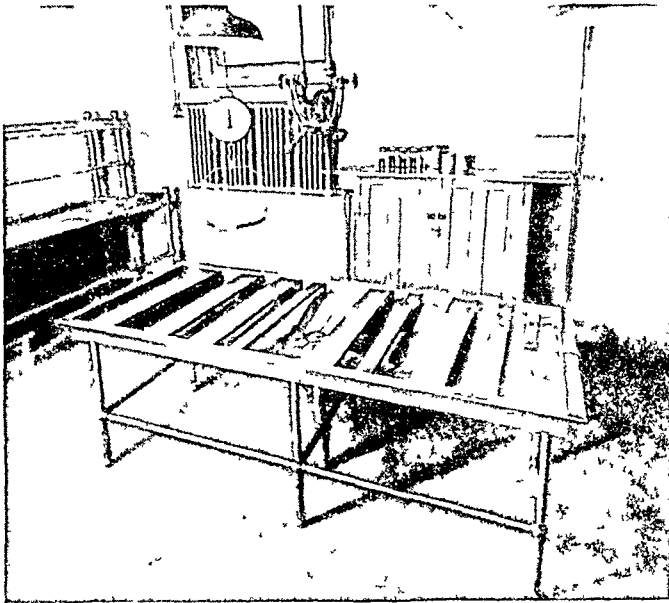
The shallow pan is fitted with eight cross-pieces or slats made of wood and entirely covered by monel, with the seams soldered on the under side so that there are no open gaps through which infectious material may creep. These slats rest on short legs also covered by monel, and their upper surfaces are  $\frac{1}{2}$  inch (1.27 cm.) below the surface of the frame of the table. Of the slats, five are 5 inches (12.7 cm.) in width and three are  $2\frac{1}{2}$  inches (6.27 cm.) in width. The two sizes of slats are convenient in that the smaller ones offer support where comparatively little is needed, without covering up too much of the underlying pan. The larger ones are used where greater support is needed, as under the shoulders or buttocks of a heavy body, or they can be placed close together to make a cutting or examining surface on the top of the table. These eight slats seem to comprise the right number. They offer all the support needed, without covering up too much of the underlying pan. When placed tightly together they cover less than half of the table, so when they are arranged, more than half of the pan is open from above for drainage or for the washing of organs or sponges. These slats are easily sterilized either by being washed with disinfectants or by being removed entirely and placed in an autoclave.

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1. Robertson, H. E.: A Post-Mortem Table (unpublished communication), read before the International Association of Medical Museums, March, 1929, Chicago.

Above this table is the pipe for the delivery of water to the table, and to this is attached a suction pump. At the head of the table is a clamp holding a head-rest, which can be quickly removed or adjusted to any height, and turned either over the table or projecting out beyond the end of it.

This autopsy table has many advantages. The monel metal does not stain or discolor as easily as white, and yet, unlike zinc or iron, which turns dark and appears to be dirty, it always looks clean and bright, and a little rubbing makes it shine like a silver plate. There is no part of the table that can harbor infectious material, and it is easily sterilized. It is light and easily movable except for the attachment of the central drain. It is long enough to allow at the foot a wooden stand for the cutting of organs or for the placing of extra instruments or solutions. It is wide enough to accommodate a large body, or if necessary, two bodies, and to allow many people to work around it. There is free drainage from any one part of



Autopsy table with head-rest and slats in place ready for use.

the body into the underlying pan, without soiling or contaminating any other part of the body. For example, pus from an abscess will drain directly down into the pan without running over or about the body. The adjustable cross-pieces give sufficient support in the right places for either an infant or an obese man. Removed organs may be dropped into the underlying pan, out of the way, where they will be automatically washed by the running water until they are needed or discarded. Also, the pan, being lower than the surface of the table, prevents the usual splashing or spraying of fine particles of fluid, which may or may not be infectious, from the surface of the table. This is of real value in preventing contamination of the clothing of the spectators, who may go from the autopsy room to other parts of the hospital or to visit patients. Also, on this table, an autopsy can be made with the minimum amount of soiling or discoloration of the body by blood or fluids, and consequently it will offer much less offense to the spectators and will be a factor in destroying an aversion to autopsies, which will help to obtain more autopsies.



# General Review

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## EXPERIMENTAL STUDY OF TRAUMATIC SHOCK

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CHICAGO

The present review was undertaken to ascertain, primarily, the trend of experimental investigation on the ever current subject of traumatic shock. Mann in 1914 came to the somewhat discouraging conclusion that "despite the enormous amount of work which has been done on surgical shock there is still among recent writers no general agreement as to the causes and nature of this condition. A critical review of the literature reveals an astounding amount of contradictory experimental data, and a great number of diverse conclusions based thereon." He found no common standard of shock. "In protocols of some observers a markedly lowered blood pressure was taken as the sole indication of the presence of shock. In others it seemed to have been thought sufficient to state—'after reducing the animal to a state of shock,' without giving the methods of its production or any criteria by which it is possible to judge whether an animal really was or was not in a condition of shock. This lack of definiteness in regard to these fundamental matters made many of the most extensive researches on shock of somewhat questionable value." Cope in 1928 was dissatisfied with popular conceptions of shock and found eight textbook definitions of it inadequate or ambiguous. Some of them obviously were definitions of collapse; some were couched in such terms as to make shock and collapse synonymous. Cope deplored the fact that most of these definitions were based on a consideration of traumatic shock alone and argued for the adoption of a broad point of view—"just as fever is so considered. Shock is a depression, as fever is generally an exaltation, of the phenomena of vital activity." He maintained, ". . . the term shock signifies a condition following the application of harmful stimuli (traumatic or toxic) or the depletion of the body fluids, in which there is clinically demonstrable depression of the vital processes of the body, particularly the circulation and metabolism. Collapse is the term applicable to the sudden onset or rapid aggravation of the symptoms of

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From the Norman Bridge Pathological Laboratory of Rush Medical College of the University of Chicago.

shock." Mann favored a definition that does not involve any more or less questionable theory of etiology, such as that suggested by Meltzer. "Shock is a state of general apathy, reduced sensibility, extreme motor weakness, great pallor, very rapid, small pulse, thready, soft arteries, irregular gasping respirations and subnormal temperature."

Confusion in delimiting shock is reflected in the numerous theories of its origin. Discussion of these theories and details of their individual merits are abundant in the voluminous literature on traumatic shock (Mann, Cope, Risley, Macleod, Cowell, Parsons and Phemister). The favorite procedure in many publications, of a preliminary discussion of theories, has been justified by the astounding discrepancies between theory and fact later disclosed by huge series of well controlled experiments on animals, laboratory tests and clinical observations. The chaotic impression created by an ensemble of the results of investigations on shock is lacking in many individual researches which are replete with evidence of ingenuity, resource and logical deduction. Gradually a more accurate appraisal of the phenomena of shock has resulted in a grouping into essential and auxiliary features. Simplification of the concept of shock has brought into prominence the basic character of alterations in the volume of the blood in the production of this condition. Proof of the importance of this factor in the production of shock is intimately coupled with disproof of the importance of other factors. In tracing the evolution of modern notions of shock, I have found it helpful to resort to somewhat detailed experimental evidence for and against the various theories.

#### THE VASOMOTOR EXHAUSTION THEORY

Because of the marked physical and mental depression occurring in shock, exhaustion of vital nerve centers was held responsible for the condition. Painful stimuli arising from a wound were thought to produce the exhaustion. Crile (1923 [b]; 1922) has been the most ardent exponent of this line of reasoning and has demonstrated morphologic and conduction changes in the tissues of rabbits following exhaustion produced in various ways. He pointed out that all cells are composed of two colloids separated from each other and from the outside by nuclear and cell membranes. The nuclear material is acid in nature; the cell body, alkaline. The nucleus of a cell as compared with the cytoplasm is positive. Each cell is therefore an ultimate electrochemical unit, and each organ and tissue an electrochemical mechanism. The normal conditions in the body obtain when the brain acts as the positive pole and the liver as the negative pole, and when adequate oxygen and epinephrine are available. On the basis of conduction measurements he assumed that the first effect of stimulation within the organism is

a slight decrease in the conductivity of the cerebrum followed by a gradual continuous decrease. The results indicate that the intracellular changes in excitation and exhaustion that are revealed by the microscope are paralleled by alterations in electrical conductivity, and that both the histologic and the electrical changes bear a direct relation to the vitality of the organ. When differential stainability of the nerve cells is lost, these cells have ceased to be efficient batteries.

Dolley described morphologic changes in nerve cells in shock and severe anemia and found these changes identical in the two conditions. He was of the opinion that the cerebral cells had no chance or means of recuperation and, driven by never ceasing stimulation, ran their life cycle in a few hours. A definite sequence of alterations in the relation between the extranuclear and intranuclear chromatin material was believed to represent functional activity carried to its ultimate limit. The essence of the process, so he said, was the continued overproduction of chromatic material up to the limit of capacity, followed by using it up from this maximum point down to its complete disappearance from cell body and nucleus. He found that these alterations were most clearly evident in the Purkinje cells of the cerebellum and were observed in three stages. In the first there was a disappearance of extranuclear chromatic material; in the second, a using up of diffused intranuclear chromatic material after it passes in turn into the cytoplasm, leaving the karyosome intact, and, in the third, the giving up of the chromatic material by the karyosome which, after diffusion into the cytoplasm, also died. By means of camera lucida drawings, cell volume and nucleus-plasma ratios were accurately determined.

Confirmation of these cellular changes in the central nervous system is supplied, in some measure, by Mott, who studied the brains of soldiers dead from shell shock, with and without burial and with probable gas poisoning; the brains of three men dying of wound shock; the brain of a person who died of shock following extensive burns; brains removed after death from anaphylactic shock; the brain of one who died of shock following contusion of the heart; the brain of a man who died under anesthesia, and brains of animals which had been given injections of histamine. The material acquired from such varied sources had two classes of alterations in common, chromatolytic and biochemical. Routine sections were made of medulla in order to study the vital centers, and of cerebellum because of its uniform structure. In the brains of bodies dead from shock there was a definite diminution of basophilic substance and a diffuse purple-staining of the cells.

Opposed to these views that exhaustion is an initiating factor in shock are others which indicate that it is of relatively little importance. Janeway and Ewing were unable to produce shock by afferent electrical stimulation for two hours in the presence of a good blood pressure

and unimpaired vasomotor compensatory mechanism. Mann performed two crucial experiments to show that shock produced by the easiest and most certain method, traumatization and exposure of the abdominal viscera, is not due alone to paralysis of the vasomotor mechanism of the splanchnic area. By section of the spinal cord or of the splanchnic nerves he was not able to produce shock. By removing the thoracic and abdominal organs from an animal, the "visceral organism" could still be kept alive many hours, and function. Therefore he concluded that some cause other than vasomotor paralysis or inhibition was involved in the production of shock. Cannon (1922) transected the spinal cord above the lumbar plexus and severed all the nerves to a cat's hind limb before traumatizing it with a hammer, but was unable by these precautions to prevent the development of shock. In his experiments he found no evidence that impulses caused by trauma might produce reflex inhibition of the vasomotor center.

Porter, working on cats, found that stimulation of the depressor nerve caused a 46 per cent fall in blood pressure. After this animal lay in shock for eight hours, with exposed intestines painted with nitric acid, stimulation of the depressor nerve still caused a 45 per cent fall in blood pressure. In order to test the effect of frequently repeated stimuli over long periods, the depressor nerve of a cat was electrically stimulated at intervals for eight hours. The initial stimulation, with a current of 1,000 Kronecker units, produced a fall equal to 35 mm. of mercury. A final stimulus of the same intensity caused a fall of 42 mm. Other experiments on cats near death from pneumonia and on a rabbit immediately after it had died of diphtheria proved the normal reactivity of the vasomotor center. Porter pointed out that since the depressor nerve can affect the blood pressure only through the vasomotor center, the vasomotor center is not exhausted in shock. He concluded:

Experimental evidence shows that failure of the vasomotor center is due to anemia rather than stimulation. If the blood pressure in the nutrient capillaries falls below the critical nutrient level—a very low level—the vasomotor cells are at once affected. Bulbar anemia may be a consequence of prolonged shock especially when the bulb is allowed to be higher than the trunk, for a man with shock is a gravity animal and his cerebral circulation then depends chiefly on small differences in hydrostatic level, but an injury after shock is not to be confused with an injury causing shock. In short, exhaustion of the vasomotor center is a taking phrase but it is nothing more.

#### THE FAT EMBOLISM THEORY

In 1916, Porter was informed by the French military surgeons at the Carrel Hospital in Compiègne that shock occurred most frequently after shell fracture of the femur and after multiple wounds through

the subcutaneous fat. He verified this statement soon afterward from observations on over one thousand freshly wounded soldiers, and then proceeded to prove experimentally that fat embolism might cause shock. Curiously he found that "notwithstanding the very numerous clinical and pathological studies of fat embolism there has been, heretofore, no attempt made to demonstrate by measurement of the blood pressure a causal relation between fat embolism and the shock of the battlefield." By injecting fatty substances such as olive oil and thick cream into the jugular veins of cats he was able to reproduce in them the clinical picture of shock. In order to exclude embolism of the lungs and heart, Porter curarized a rabbit, instituted artificial respiration, ligated the subclavian artery just after its exit from the aorta and distal to the vertebral artery and tied off all but two muscular branches. With the vertebral and two muscle arteries the only ones patent, he then injected 0.1 cc. of oil per kilogram of body weight. In fifteen minutes, the carotid diastolic blood pressure had fallen from 160 mm. of mercury to 20. Fat was hard to find in sections of the vasomotor center stained with scharlach r when such small quantities of oil were employed. The physiologic evidence in favor of fat embolism is, however, convincing because all portions of the brain in a cat may be excised without lowering the blood pressure so long as the vasomotor center is left intact. Thus Porter attributed the fall in blood pressure after fat embolism to injury of this center. As some experimenters had been unable to produce a fall in blood pressure by injecting fat into the peripheral end of the carotid artery, they concluded that the important features of fat embolism were associated with embolism of the lungs rather than the brain. Clinging steadfastly to this theory of fat embolism of the vasomotor center, Porter pointed out that the vasomotor center derives its blood supply from the basilar artery and not from the carotids. He recognized the rich anastomoses between branches of these vessels in the circle of Willis, but believed that this circle is a balanced pressure ring in which pressure from each carotid region contends with that from the basilar region. Thus oil injected into the carotid arteries seldom caused shock because it entered parts of the brain anterior to the bulb. If, however, one carotid artery was clamped and oil injected into the other, shock usually occurred. When both carotid arteries were tied, the vertebrals left patent and oil injected into the external jugular vein of a cat, shock usually took place. When, on the other hand, the vertebral arteries were tied and both carotids were free and oil was injected into the external jugular vein, shock seldom occurred.

Under the conditions of the last two experiments, the lungs were embolized equally in both series, but since the results were decidedly different, pulmonary embolism could not have been of great importance.

The interpretation of these results by Porter was again embolization of the vasomotor center via the vertebral arteries.

Mott in his studies of brains from soldiers who died in shock found fat emboli in the medulla of two who had shell-fractured femurs, but in no others was fat evident. This corroborative evidence of Porter's fat embolism theory is, however, exceedingly scanty as compared with the numerous objections to it raised on theoretical, clinical and experimental grounds. Wieting in his extensive review of the literature ascribed no importance to either fat or air embolism in the causation of shock. Cannon (1922) found the fat embolism theory defective in that it offers no adequate explanation for the onset of delayed or secondary shock. It also fails to account for the occurrence, both in clinical and experimental shock, of a diminution of blood volume and either a local or a general concentration of corpuscles. Parsons and Phemister found fat in the lungs of each of ten dogs the limbs of which had been hammered. In several this was slight; in three, moderate. The central nervous systems of four dogs were examined for fat after trauma, and in three of the dogs, bones were intentionally broken to favor the development of fat embolism. There was practically no fat in the blood vessels of the cerebrum, cerebellum or medulla, and so these authors concluded that fat was too scanty to have played a rôle in the production of shock.

#### THE ACAPNIA THEORY

The acapnia theory of Y. Henderson (1908) was regarded as promising before attempts were made to ascertain the relative importance of the different alterations that, taken altogether, constitute shock. According to Henderson, exhalation of carbon dioxide is responsible for the development of apnea and eventually respiratory failure. This exhalation may result clinically from a violent and prolonged hyperpnea caused by stimulation of afferent nerves during injury. It may also occur when the abdomen is opened in surgical operations, even when cooling and drying are prevented. The marked hyperemia of the viscera thus exposed, even when no further procedures are undertaken, is presumably due to loss of carbon dioxide and is relieved by placing the part in saline solution saturated with carbon dioxide. Experimental hyperventilation of the lungs may result in a shocklike condition. The theory acquired support from studies on gas tension in the blood and tissues, especially studies of mountain sickness. In this connection, Henderson cited the work of the Italian investigators, Mosso and Marro, who found by blood gas analyses that when animals were transported up to the physiologic laboratory on Monte Rosa or placed in the low pressure chamber at Turin, the carbon dioxide content of their arterial blood was reduced. Unlimited quantities of pure oxygen

were incapable of averting muscular weakness, sleepiness and, at times, almost vomiting in animals subjected to decreased barometric pressure.

This attractive, interesting hypothesis of Henderson's was soon confronted by the results of experiments by a number of investigators. Mann prevented escape of carbon dioxide from the abdomen of the dog by making a small incision, inserting his gloved hand and then clamping the skin of the animal tightly about his wrist before traumatizing the viscera. In other experiments he passed a stream of gas containing a high percentage of carbon dioxide into the abdomen. Despite these precautions, the animals went into shock. Janeway and Ewing instituted artificial hyperventilation in dogs up to seventy breaths a minute, produced a fall of up to 50 per cent of the carbon dioxide of the blood in one-half hour and had the animals in shock in from two to three hours. The dogs had interstitial emphysema of the lungs caused by the excessive pressure necessary for this type of experiment. Still more subversive for the acapnia theory were their experiments in which they provided a rebreathing apparatus for carbon dioxide or kept the pressure constant from a tank of this gas and achieved results identical with those in which such provisions were lacking. They were thus forced to disregard diminution in carbon dioxide as the cause of shock. By employing simultaneously a cardiometer in circuit with a recording tambour, a water manometer, connected with a bronchus and a blood pressure cannula in the carotid artery, they proved that increased intrathoracic pressure was the all-important factor under the condition of hyperventilation. This increased intrathoracic pressure hindered venous return to the heart by direct pressure around the pulmonary artery and on the capillaries and veins within the lungs themselves. In an experiment, an increase of intrabronchial pressure from 8 to 30 mm. of mercury caused a drop in blood pressure from 122 to 55 mm. of mercury and a 44 per cent diminution of cardiac output. A rise of intrabronchial pressure above 8 or 10 mm. of mercury always caused a fall of blood pressure proportional to the rise of intrabronchial pressure. As an improvement over previous investigations on abdominal ventilation, these men sewed a celluloid window between the recti muscles so that they could observe the viscera and control aeration with warm, moist air. When intestinal peristalsis was almost absent, the blood pressure was still 163 mm. of mercury. After removal of this window and aeration for forty-five minutes longer, the blood pressure was 153 mm. of mercury and the carbon dioxide of the arterial blood 38.8 per cent by volume. After the intestines had been handled for ten minutes, the blood pressure was 80 mm., and after twenty minutes, 56 mm. of mercury. At the end of thirty minutes, the carbon dioxide was 31.6 per cent by volume. Still other experiments were performed in which the carbon dioxide in the blood was kept at a normal level

through a long tube inserted into the trachea. After aeration of the intestines for one and one-half hours with the abdominal wall and omentum removed, the blood pressure remained unchanged and the animal was in good condition. At the end of this time, the intestines were handled, and in ten minutes the blood pressure fell from 122 to 60 mm. of mercury, although the carbon dioxide content of the blood was 45.1 per cent by volume. In twenty-five minutes, the blood pressure was 46 mm. of mercury, the carbon dioxide was undiminished, and the dog was in profound shock. Stimulation of the sciatic nerve at this time caused the blood pressure to rise to 96 mm. of mercury, indicating a strong medullary reaction.

#### THE ACIDOSIS THEORY

The carbon dioxide content of the blood in shock has also been studied thoroughly from another angle—its possible relationship to acidosis as the cause of shock. Controversy on this point soon subsided when investigators qualified the term of acidosis in the sense of L. T. Henderson and Van Slyke—a diminished alkali reserve rather than a marked change in hydrogen ion concentration. Studies of the alkali reserve have been made as a routine by many experimenters, regardless of what features of shock they were concerned with or to what theory they subscribed. The result has been a concurrence of opinion, an all too rare situation on almost any phase of traumatic shock. The researches of the British Shock Commission helped to establish the precise relationship of acidosis to shock. The first of a series of experiments consisted in injecting 30 cc. of normal lactic acid into the internal saphenous vein of a cat weighing 3 Kg. in a period of twenty-four minutes. Before the infusion, the combined carbon dioxide content of plasma removed from the jugular vein was 46.4 per cent by volume; during the last minute of infusion, it was 10.3; ten minutes later, 22.25, and one hour later, 32.1.

The animal was not in shock after intravenous injection of an amount of acid far in excess of any that would be formed in its muscles after fatigue or injury. Since acidosis produced in this manner was more simple than that which might occur in shock following wounds, acidosis was combined with hemorrhage, with injections of histamine, epinephrine hydrochloride and peptone and with tests of activity of the vasomotor center. In no instance did acidosis modify the action of the other agents in reducing the carbon dioxide-combining power of the blood. It was found that hemorrhage severe enough to reduce the blood pressure to 79 mm. of mercury caused rapid and serious decline of the alkali reserve. A cannula tied into the pericardial sac and connected to a buret filled with physiologic solution of sodium



chloride served as a variable system for restricting the filling of the heart. By simply raising or lowering the reservoir, pressure within the sac could be maintained at any desired level, and so various blood pressures could be affected. When a blood pressure of 80 mm. of mercury was maintained in the animal's arteries, no ill effects were noted, but when this was forced down to 60, acidosis developed. If, however, 20 per cent of the animal's blood was removed by bleeding, a reduction of its blood pressure to 80 mm. was sufficient to cause acidosis. The normal average alkali reserve of the dog's venous plasma is 43.4 per cent by volume, with variations ranging from 32.4 to 59.5. Raymond found that the mean alkali reserve fell to 26.1 if ether anesthesia was maintained for from forty-five minutes to two hours, but that the mean fall decreased per unit of time after this period. In some dogs, the alkali reserve fell no lower in profound shock than in experiments of similar duration under ether anesthesia control performed on the same animals a week previously, from which they completely recovered. He could not discover a critical level of alkali reserve. Cannon and Cattell, using an intrapericardial cannula, were convinced that the critical level in blood pressure, below which the alkali reserve diminishes, is 80 mm. of mercury. The unanimity of opinion as to the occurrence of a diminution of alkali reserve in shock is not evident in the explanations offered for its development. The oldest view that a nonrespirable acid such as lactic acid develops in the tissues because of a want of oxygen, unites with the sodium of the sodium bicarbonate and then drives off the carbon dioxide, which is breathed out, was termed by Cannon the acidotic theory. Y. Henderson saw in this diminished alkali reserve support for his acapnial theory.

The process viewed in this light was based on the assumption that excessive respiration diminishes the carbonic acid in the blood with consequent alkalosis, and that thereupon the extra alkali disappears into the tissues or diffuses into the body fluids outside the blood or escapes through the kidneys. An explanation, based on experience with gas bacillus infection, was offered by Wright. He proved that in its growth *B. welchii* diminishes the antitryptic power of the medium and renders it acid. These chemical changes furnish the conditions required for the avalanchelike inroad of the bacterial infection. Experiments by the Medical Research Committee proved that it is the toxemia of the gas gangrene type that produces the acidosis rather than a change in reaction in the tissue fluids. In a specific experiment, 20 cc. of alkaline fluid from the edematous muscles of a puppy infected with the vibriion septique was injected into a vein of another puppy during a period of twenty-five minutes. Although the fluid that had been injected was alkaline, fifteen minutes after the injection the puppy's blood pressure had fallen from 116 mm. of mercury to 64 and the alkali

reserve from 43 to 27 per cent by volume. Half an hour later the blood pressure was 30 and the alkali reserve 7. Cannon is supported by several investigators (McEllroy, Erlanger and Gasser, Gesell, Penfield) in his contention that all conditions associated with an increase of lactic acid or a reduced alkali reserve are characterized by diminished blood volume or diminished blood pressure or decreased carrying power of the corpuscles, so that delivery of oxygen is reduced.

B. Moore also tried to combine the various views on diminished alkali reserve on an acapnial basis. He assumed that as a consequence of shock there is a general lowering of metabolic activity to about one third of the normal rate. If then the lungs continue to function at their usual rate, or even less, there will be an excess of carbon dioxide removed from the blood, and the blood will become more alkaline. Then the kidneys and tissue cells will remove alkali from the circulation, and the blood then examined will have less sodium bicarbonate in it than normally. The excessive breathing held necessary by Henderson and Haggard for the development of acidosis is, according to Cannon (1922), not present in wounded soldiers, whose respirations in shock are shallow, feeble and interrupted by occasional sighs.

Aub studied the basal metabolism of cats under ethyl carbamate anesthesia, and found an increase in pulmonary ventilation of 54 per cent after the hind limbs had been crushed, but before the onset of shock. He found that the basal metabolism decreased 19 per cent after mild shock and 30 per cent after severe shock. Increase of intrapericardial pressure to reduce the blood pressure to a critical level of 75 or 80 mm. of mercury caused an average decrease in metabolism of 31 per cent. The prompt reduction of metabolism by this method of merely hindering the venous return to the heart is evidence that some mechanical factor such as retarded blood flow is the cause of reduced metabolism. Aub was certain, however, that reduced blood pressure alone does not account for the fall of metabolism, because a low blood pressure following hemorrhage may be associated with only a slight drop in metabolism. After muscle injury, the metabolism may be reduced before a great fall in blood pressure has occurred. A possible explanation of these discrepancies is offered by Gesell, who found that in the early stages of shock produced by injury of tissue there is usually a marked reduction in the volume flow of blood in the peripheral organs, although the blood pressure is only a little changed. The volume flow of blood determines the delivery of oxygen to the tissues, and this may vary to some degree without a corresponding variation in the blood pressure.

#### THE TOXEMIA THEORY

The resemblance of a patient in traumatic shock to one prostrated from some overwhelming toxemia has been stressed repeatedly and

investigated extensively. In certain patients, e.g., those with gas bacillus infections, the connection between toxemia and shock was evident. In the great majority of persons suffering from shock during the war this condition developed before they could have been infected with gas-forming organisms. The thought of such an infection is not tenable in the case of civilians or that of laboratory animals. Some common factor producing the symptoms of shock in animals and men after injury was demanded. One result of efforts to answer this requirement properly has been the attention directed to the refuse of damaged tissues. The proteins and their cleavage products were suspected of exerting such an influence when abnormally liberated in the body, because of numerous physiologic researches with peptone. Vincent and Sheen, using saline decoctions and proteid, alcoholic, and ether extracts intravenously in cats, dogs and rabbits, noted a marked depressor effect of such extracts. Toxic substances were found in the fluid in a closed loop of bowel by Whipple, Stone and Bernheim, who were concerned with intestinal obstruction. The toxic element of fluid from a closed intestinal loop was found to emanate in the mucosa. The crowning achievement in production of shock by the injection of toxic agents was the use of histamine by Dale and Laidlaw. Shock produced by this substance could be varied according to the amount employed and the time allowed for its administration. In guinea-pigs and rabbits, small doses produced phenomena usually associated with anaphylactic shock. Sudden introduction of a large dose resulted in effects divisible into three phases: The first was marked by an immediate fall in blood pressure of about 60 mm. of mercury, due to constriction of the pulmonary arterial branches and a diminished output of the left ventricle of the heart. The second phase, which, like the first, lasted only a few seconds, was marked by an arrest of the downward tendency of the arterial pressure and by a secondary rise due to the stimulating action of the drug causing vasoconstriction and generalized contraction of smooth muscle. Finally, about four minutes after injection of this poison, symptoms of shock set in.

To approximate more nearly conditions in which shock is seen clinically, Cannon and Bayliss bruised the muscles of a cat's thigh and noted a prompt fall in blood pressure. This fall failed to appear if the aorta was clamped above the bifurcation during the trauma, but was not averted by transection of the spinal cord in the lumbar region. The fall, therefore, was presumably due to a combination of dilation of blood vessels in the injured area, together with some extravasation of blood, and the rapid setting free of a chemical substance acting as a powerful depressant on the blood pressure: The secondary fall in pressure was explained by the continued absorption of the depressant substance and by the establishment of a vicious circle in consequence

of the low blood pressure. Subsequently Cannon (1922), by elaboration of this experiment, sought to establish a connection between local injury of tissue and the general bodily condition through the circulation. Usually when the muscles of the hind leg of a cat were crushed, shock developed in about twenty minutes. When, however, the femoral artery and vein were ligated prior to trauma, shock did not develop in even thirty-three minutes. When the ligatures were removed from the vessels there ensued a marked fall in blood pressure, which diminished after the vessels had again been ligated. When a constant level in blood pressure had been reached, massage of the crushed muscle produced a further drop in blood pressure, a discovery that had a practical bearing on the immobilization of bony fragments. Whatever the nature of the substance in the damaged tissues, it seemed to be fairly promptly changed in the body or eliminated.

Obviously, most of the evidence for a toxic cause of traumatic shock obtained from experiments has been based on the use of histamine or on the assumption that a histamine-like substance is formed in the injured tissues. Histamine has been isolated from the dog's intestinal mucosa (Dale). Search for other sources of toxic material has also borne fruit. A toxin seems to be liberated by the breakdown of platelets from the blood of horses, rabbits and dogs. Greeley obtained quantities of this toxin sufficient to kill the animal from a number of platelets that it is easily capable of providing. He stated his belief that trauma to the labile platelets during accidents and operations may result in their disintegration. He was not in accord with von Behring, who found clumped platelets in the cerebral vessels in anaphylactic shock and attributed the phenomenon of such shock to their presence. Dale and Laidlaw found large clumps of agglutinated platelets in films of blood from animals in histamine shock. Greeley guarded against thrombosis in some of his animals by repeatedly defibrinating large amounts of their blood and then reinjecting it and also rendered the blood incoagulable by injections of heparin. His platelet extract under such circumstances was still capable of producing shock and death.

Extensive experience with wounded men in shock led many surgeons to suspect a toxic factor in the development of shock. Quenu summarized the observations of French surgeons on this subject in the following way: Secondary or traumatic shock does not appear immediately after the receipt of wounds, and therefore is not due to a nervous effect. Shock is frequently well established before infection has set in; therefore it is not of bacterial origin. Shock is most frequently seen after extensive damage of muscles or after multiple wounds. Support for this view was given Quenu by the Medical Research Committee after determining that there was an increase in undeter-

mined nitrogen, total nonprotein nitrogen and residual nitrogen in the blood of patients in shock. This was accounted for as a consequence of the absorption of material from the traumatized area and also as perhaps due to the effect of damage done to tissue by circulating toxins. Recently studies of metabolism following reduction of high blood pressure have suggested a much simpler explanation for these disturbances in body chemistry and will be referred to later. Quenu noted also that factors favoring absorption at the region of injury favored the production of shock. Thus shock was most severe when the region of damage communicated with the exterior by only a small orifice. The negative aspect of this evidence was presented by soldiers in whom a large fleshy mass, together with the skin that covered it, was carried away by a missile; in these soldiers very slight shock developed, or none. Anything that delayed or checked absorption from the injured region delayed the development of shock, but if there was a sudden removal of this check serious results followed. Thus war surgeons saw shock develop rapidly after removal of a tourniquet from a crushed extremity or after the clearing of timbers and débris from a pinioned limb.

Suppression of the injured area, if not too long delayed, caused shock to disappear. Thus McKee and Wallace and their co-workers noted the marked improvement that followed removal of a mangled limb and were able to keep the mortality down to 11 per cent if operations were performed within three hours of the injury. Amputations performed eight hours after injury were attended by 75 per cent mortality.

Attempts to cross the circulation of an animal in shock with that of a normal animal have been made with various results. Crile (1923 [a]) was unable to produce shock in a normal dog in which the carotid artery and the internal jugular vein were anastomosed to a dog in which shock was produced by intestinal manipulation. Bazett and Quinby managed to cross the entire systemic circulation from one animal to another by means of an anastomosis made at the level of the ascending part of the aorta. In experiments on shock, they found that the animal in better condition bled into the other, so that no definite evidence could be obtained. Janeway and Ewing also found that their experiments were unsatisfactory because the recipient drained off so much blood that the donor became exsanguinated. McIver and Haggart avoided the difficulties experienced by previous investigators by crossing only the circulation from the traumatized lower extremities of one cat to the intact cephalic half of another cat. The caudal half of this second cat was amputated to prevent the flow of blood through the large veins in the spinal canal. With this method shock developed

in nine of the twelve cats that had taken over the circulation from the traumatized region. In the three instances in which shock did not develop, the experiments were discontinued at the end of one and one-half hours.

Quenu stated that blood from an animal in shock will, on intravascular injection, cause shock in a second animal. Parsons and Phemister found alcoholic extracts of blood extravasated into traumatized limbs produced, on intravenous injection, a fall of blood pressure no greater than that produced by an extract of the same amount of normal blood. They investigated various possible toxic elements of shock in forty-five dogs the extremities of which were traumatized, but with consistently negative results. Thus they found only minute traces of hemoglobin in the plasma of blood drawn from the jugular vein. This was evidence against the view that damaged, extravasated or hemolyzed blood in the traumatized limb might enter the circulation and bring about a fall in blood pressure. Blood from the femoral vein of a traumatized limb was collected, without shaking, in a viviperfusion flask and then circulated through the femoral artery of a normal dog without producing vasodilation in the limb or a fall in general blood pressure. In order to determine the presence of toxic substances in devitalized muscle, the entire right rectus muscle was excised aseptically and reimplanted in three dogs, intraperitoneally in two, and into its bed in one. The animals were observed for three weeks after operation, but showed no signs of intoxication.

Because of the many experiments in which shock was produced by intestinal trauma and in which resulting visceral hyperemia ensued, the idea of splanchnic engorgement in shock produced in other ways was prevalent. Wallace and other surgeons were convinced, however, that splanchnic engorgement is not present in shock, after they had performed many hundred laparotomies on wounded soldiers. R. M. Moore determined the state of contraction of the spleen in various stages of traumatic shock, and in every instance found the spleen so extremely constricted that surfaces made by cutting did not bleed. The spleens were found to average about 0.25 per cent of the body weight—a weight that corresponds to the minimum for a completely contracted spleen. Moore regarded the spleen as a large and modified splanchnic blood vessel, the volume of which serves as an index of the state of contraction of the splanchnic vascular bed as a whole.

The occurrence of extreme contraction of the spleen in traumatic shock is interpreted as supporting the observations of other investigators to the effect that the splanchnic vasomotor mechanism becomes highly active in shock in an attempt to maintain an effective blood pressure in the presence of rapidly diminishing blood volume.

## CAPILLARY CHANGES IN TRAUMATIC SHOCK

With the development of newer ideas on capillary circulation the importance of the capillary stasis of blood in traumatic shock was emphasized. The direct observation of capillaries by such investigators as Krogh, Landis, Densham and Wells, Hooker, and Hill and McQueen revealed the potential vastness of the capillary bed. It is now generally conceded that not all capillaries are actively engaged in circulatory activities at all times. It was assumed by some of the earlier observers that those capillaries not filled with blood were too small to admit blood cells, but served only for the transport of plasma. More recently the interpretation applied to these closed vessels is that they represent the reserve irrigating channels that are opened only when the muscle is actively contracting. In muscles at rest there is a definite regularity in the distribution of patent capillaries, just sufficient channels participating in the circulation to satisfy the metabolic requirements. The distance between capillaries apparently is almost constant not only for resting and active stages in general, but also for a given group of muscles. Thus the capillaries in a frog's rectus abdominis muscle are spaced at intervals of 60 microns, whereas in most of the muscles of the trunk the open capillaries are from 200 to 500 microns apart. Permanent preparations of resting and active muscle have been made by Krogh after the capillaries had received an injection of india ink. Another observation made by him was that frog's corpuscles frequently pass through capillaries smaller than the smallest dimension of the corpuscle by a process of lateral folding or longitudinal stretching. When the muscles are active, their capillaries dilate and allow freer passage of corpuscles.

The oxygen pressure in muscles at rest is very low, but in working muscles it approaches very near to that of the blood (Densham and Wells). Although the normal tension of oxygen in the capillaries is small, insufficient oxygen for even short intervals has disastrous effects. Immediately after a three-minute period of oxygen lack, fluid filtered through the capillary wall at approximately four times the normal rate. This increased permeability of the wall also permitted the passage of protein, reducing the effective osmotic pressure of the plasma proteins to almost one-half their normal value. Exposure of the frog's mesentery to Ringer's solution, one-half saturated with carbon dioxide, produced no change in capillary fluid movement, while complete saturation increased the rate of fluid movement very slightly, but the wall remained normally impermeable to protein.

Increase of hydrogen ion concentration produced almost no change of fluid movement within physiologic limits, though at  $p_H$  4 the characteristic effects of injury occurred. Hill and McQueen noted the

existence of direct anastomotic vessels between capillaries and venules. If no such vessels existed, they maintained, the slowing in the blood current in the capillary area would uniformly lead to concentration so great that little or no blood would be in the circulation. Normally, differences greater than 3 per cent do not exist between venous and capillary erythrocyte counts. This is assumed from the usual conception of the circulation as passing from the arterioles through the capillaries into venules and then into veins. In traumatic shock in men there are not infrequently 2,000,000 fewer red blood cells per cubic millimeter in the venous blood than in that from capillaries. The anastomotic channels would, under such circumstances, maintain a certain minimum amount of blood in circulation. When eventually capillary stasis in shock reduces the available circulation through these channels, disaster results.

Cannon, Frazer and Hooper noted marked capillary stagnation and high red cell counts in severe shock and found that this condition once established was only gradually recovered from. Restoration to normal venous and capillary blood cell ratios often required three days. These observations were evidence in favor of the view that the circulatory difficulty in shock is due to loss of blood from the circulation, though not from the body. This withholding of blood from the normal currency explained most easily the existence of shock in men who had not bled. Such a conception of shock was a radical departure from the original one, and Cannon suggested the hippocratic term "exaemia"—drained of blood—as being more descriptive. Cannon is inclined to agree with Cowell that primary shock, coming on immediately after a wound, is best accounted for as the result of nervous action. Fainting after slight wounds is a transient state of this nature, which in true shock is more persistent. A combination of facts to explain secondary shock and stress exemia was accomplished in the following fashion: Sweating and exposure lead to rapid loss of heat from the body, and low external temperature favors the process. Inactivity of the wounded man and absence of shivering lessens production of heat. Thus the body becomes cold. In consequence of the low blood pressure aided by chilled tissues, there is stagnation of corpuscles in the capillaries. The onward flow here checked undergoes concentration, so that the capillary red cell count is high. Prolonged lack of fluid and sweating may favor the stagnation and further concentration of the blood. By accumulation in the capillaries the return of blood to the heart is lessened until a persistent low blood pressure becomes established. The blood lost from currency produces a state equivalent to hemorrhage. Any true hemorrhage therefore exaggerates the existence of shock or exemia.



## THE RELATION BETWEEN HEMORRHAGE AND SHOCK

The close connection between hemorrhage and shock has been stressed repeatedly (Mann, Parsons and Phemister). Mann was unable to differentiate between shock and the results of hemorrhage "except perhaps by a leucocyte count." Cannon, Frazer and Hooper have stressed the importance of hemorrhage in hastening the onset of shock. Blalock and his associates are convinced that loss of blood plasma is the important event that precedes the onset of shock. Their original experiments were undertaken to determine the oxygen content of the blood in shock, since lack of oxygen, as a result of decreased volume of blood, was credited with harmful effects on the tissues. Samples of blood from the right auricle, the renal, portal, external jugular and femoral veins and the femoral artery were tested for oxygen after shock had been produced in dogs by hemorrhage, injections of histamine and trauma to the cerebrum, intestines or one leg. Approximately the same relationship existed between the values of the oxygen content of the blood from various sites after low blood pressure had been produced by hemorrhage, histamine and trauma to the brain. The oxygen content of the blood from the portal vein was much higher relatively after trauma to the intestine, and that of the blood from the femoral vein of a traumatized leg was higher than that of the opposite extremity. The observations suggested a local accumulation of blood at the site of injury to a large region such as the intestine or an extremity and were evidence against the action of a histamine-like substance that produced a general bodily effect. Henderson, working with shock induced by intestinal trauma in dogs, found the oxygen content of the venous blood decreased. He attributed the effect to failure of the venopressor mechanism. Aub and Cunningham concluded that there was a markedly diminished oxygen content of the venous blood in experimental traumatic shock produced by crushing the hind legs of cats. This diminution occurred before the blood pressure fell to a shock level, and was still present after apparent recovery from shock. With this evidence of a local cause of shock to direct his efforts, Blalock (1930) hammered one hind leg in a number of dogs, thus reducing the blood pressure to a shock level, and noted that the injured side weighed from 530 to 960 Gm. more than the normal side. The increased weight of the injured limb was about 4 per cent of the body weight and was due to the extravasation of blood incident to trauma.

The importance of the loss of this amount of blood was proved by bleeding normal dogs 1 per cent of their body weight at intervals of an hour. In three or four hours, the systolic blood pressure of these dogs dropped to 70 mm. of mercury. In trauma to the intestine, the

average loss of body weight was 4.48 per cent—this mostly in the form of fluid devoid of red blood cells. In burns of one hind leg, the average increase in weight in the form of edema amounted to 3.38 per cent of the body weight. The loss of whole blood is apparently withstood better by a dog than the loss of either plasma or red cells alone. In types of shock studied by Blalock, it seemed that fluid from the blood is not reabsorbed to any marked degree because the osmotic pressure in the injured tissue is practically equivalent to that of the blood. The diminution in the capillary pressure probably tends to draw fluid into the blood stream in the noninjured regions. However, if this results, the increase in the mechanical pressure which it causes would tend to cause further loss of fluid into the injured tissues. "It is believed that the results of these experiments indicate that the loss of plasma protein is the most important factor in the production of the low blood pressure after the procedures reported." Previous work on plasma proteins (Whipple, Smith and Belt) established their great value and the fact that, once they have been depleted, their regeneration is a slow process requiring several days. Studies of plasmapheresis have proved that injured hepatic cells will predispose an animal to shock. Thus strong support is given the theory that there is a primary cellular injury that precedes the familiar clinical reaction of shock. Injuries of the pancreas, kidney and intestine do not, however, result in the development of such a predisposition when standard plasmapheresis is employed. A summary of the large number of experiments on shock by Blalock, Beard and Johnson appeared recently, and again emphasis was placed on the loss of whole blood or plasma as the major constant feature associated with shock. Parsons and Phemister found that the general symptoms developing in animals with traumatized limbs were practically the same as those that occur after hemorrhage. As an interesting incidental observation, incoagulability of the extravasated blood was reported. The reason for this behavior is not known, although search in extravasated blood and traumatized muscle for heparin failed to reveal this substance. The blood did not clot even when calcium salts or blood serum was added, although circulating blood removed from the heart before or after death clotted in normal time. Examination of the circulating blood of traumatized dogs disclosed increasing evidence of anemia which almost paralleled that in other dogs in which blood pressure had been reduced to a corresponding level.

Evidences of shock from loss of water after burns (Blalock, 1931; Underhill, Carrington and Kapsinaw; Underhill), influenza (Underhill and Kapsinaw), and a host of apparently unrelated perverted states of body, such as high fever, prolonged vomiting, as in intestinal obstruction, and severe diarrhea (Marriott), have served to stimulate investigation of the water content of blood and tissue in shock. Harris and

Blalock estimated the amount of water and solids of the liver, lung, kidney, intestine, heart muscle, brain, spleen and skeletal muscle before and after trauma to an extremity or to the intestine and after burns. There was little difference in the water or solid content after these procedures. In studies of the blood itself, a concentration of 21.5 per cent was noted after removal of plasma and after trauma to an extremity. The conclusions drawn from experiments in which depletion of plasma was produced are similar to those already arrived at by clinical observations. The plasma proteins withdrawn in the plasma or lost in a traumatized leg can no longer maintain the osmotic pressure in the capillaries or serve to counterbalance the force of filtration due to hydrostatic pressure. Loss of these colloids lowers the total osmotic pressure of the blood, and hence only a small amount of water passes into the blood stream. The same mechanism apparently is at work after hemorrhage and after shock. Associated with trauma is injury to the capillaries, which allows not only water but colloids to pass through their walls. The loss of large amounts of plasma causes a diminution in the capillary pressure, and this probably tends to allow fluid to pass into the blood stream in noninjured parts of the body as in hemorrhage. If this takes place, the increase in pressure in the capillaries probably causes further loss of fluid into the injured part. It is probably for this reason that shock due to trauma is usually more difficult to treat than that due to uncomplicated hemorrhage. After hemorrhage it is usually simply a matter of restoring the volume of the blood after the bleeding vessels have been ligated. After trauma, any procedure that increases the hydrostatic pressure in the vessels tends to cause further loss of fluid through the injured capillaries.

The latest reported investigations on the water balance in shock are those of Robinson and Parsons. They pointed out that blood is a liquid of which from 78 to 81 per cent is water, and that this water, according to recent views, is "bound" or modified by the colloids present in it. Their results are summarized as follows:

. . . in hemorrhage, injection of histamine, and shock due to hemorrhage, an alternation exists between the water content of the blood and that of the skeletal muscles. That is, under these conditions, when an increase occurs in the water of the blood, a decrease takes place in the water of the muscles, and vice versa. The changes are almost simultaneous, and in some cases seem to be proportionate. Generalized muscular activity produces slight but frequent fluctuations in the water content of both blood and muscles, sometimes increasing and at other times decreasing the percentage of water present. The alteration noted above was not observed under these conditions.

A hemorrhage of from 200 to 300 cc. of blood in dogs weighing from 10 to 14 Kg. produces an increase in the percentage of the total and free water contents of the blood. That this indicates a dilution of the blood is shown by the decrease in the erythrocyte count. A fall in the total, free, and bound water in the muscles accompanies the rise in the water of the blood.

In all dogs, after the traumatized leg has become markedly swollen there occurs an increase in the water of the blood, a decrease in that of the muscles distant from the trauma, and a decrease in the number of circulating erythrocytes indicating hemorrhage in the limb. The condition at the end of the experimental trauma shows water relations similar to those that follow hemorrhage.

#### COMMENT

The astounding variability in the results of investigations of traumatic shock can be accounted for in much of the earlier work by the variety of animals, anesthetics and procedures employed. Frogs, guinea-pigs and cats were used in the studies of capillaries. Dogs, cats, rabbits and horses were used in other phases of the problem. These animals were given various anesthetics: Some were given ether and morphine, some ether alone, and some nitrous oxide, ethyl carbamate or sodium barbital. Elaborate devices to retain body heat while procedures designed to produce shock were in progress were provided for some animals, whereas in other experiments no such provisions were made, and no record of temperature appears. The possibility that the anesthetic might alter the results of investigations of shock has occurred to many observers. Unfortunately the studies intended to settle this question have led to widely divergent views. Cattell (1923 [a]) concluded:

In normal cats, the inhalation of ether results in a sudden drop in arterial pressure which is quite temporary. As anesthesia deepens, the pressure gradually recovers until, by the time the eye reflex has disappeared, it may have returned to its original level. In the shocked animal, there is no recovery of the blood pressure after the primary fall, and the pressure continues to fall to zero even before the eye reflex disappears. Ether causes a contraction of the peripheral blood vessels due to a direct stimulation of the vasomotor center and a reflex to the fall in pressure resulting from a depression of the heart.

This cardiac depression is apparently responsible for the diminished alkali reserve that Raymond found in dogs subjected to ether anesthesia. Even more incriminating evidence against ether is supplied by Quigley and Lindquist, who were able to compress the thoracic part of the aorta by ligatures for two and one-half hours without producing shock in dogs when no anesthetic was used. Similar compression for as little as fifteen minutes under ether anesthesia, however, resulted in such marked respiratory difficulty that the ligature had to be released. Voicing a contrary opinion, Cannon did not think the anesthetic was important because his results were the same with ethyl carbamate as with ether. Cattell (1923 [b]) found that morphine does not accelerate the production of shock in cats and seems to prevent the usual reduction in alkali reserve incident to the low blood pressure in shock. Henderson argued that such conserving action by morphine is due to its depressive action on respiration.

The increase in blood nitrogen so frequently recorded in shock and assumed in many instances to be due to absorption of destroyed tissue can apparently be brought about simply by the altered metabolism that results from reduced blood pressure. The danger associated with reducing the blood pressure, even when it is far above normal, is generally appreciated in clinical medicine. The experiences of Harris and Platt are illustrative of this danger. They studied twenty-two patients with high blood pressure for a week on a rigid diet, and in the succeeding week gave them either injections of a proprietary "colloidal" sulphur solution or erythrol tetranitrate by mouth. During the period of reduced blood pressure in these patients, their excretion of water and their alkali reserve diminished, blood urea and nonprotein nitrogen increased, uric acid, phosphates and chlorides were retained, and cardiac output decreased. Such results achieved by recognized therapeutic measures indicated the importance of an adequate blood pressure in preserving metabolic equilibrium.

Wiggers used a photokymographic record obtained by a differential water manometer to study the circulatory changes in shock. He concluded that "the decreased venous pressure and consequent reduction in minute output is the predominant factor in the pronounced fall of arterial pressure during the progressive stage of shock. The dynamics of the circulation indicate clearly, however, that a reduction in peripheral arterial resistance initiates the fall of arterial pressure and the diminished filling of the arterial trunks before the effective venous pressure and cardiac discharge are reduced." These conclusions based on results obtained by the most modern instruments of precision were contradicted within a year by Erlanger, Gesell and Gasser. These dissenters, interpreting their results in terms of activity of the vasomotor center, stated that in the early stages of exposure of the intestines the tone of the center is usually increased. By the time the arterial pressure has fallen to 50 mm. of mercury, the tone of the center is invariably subnormal. The fact that vasoconstriction is the rule while the pressure is high, and that dilation occurs after the pressure has been low for some time, seems to indicate that exposure of the intestines tends to cause a fall in pressure. This tendency is combated by the vasoconstrictor center as long as arterial pressure is high enough to maintain a sufficient supply of blood to the centers. An explanation of the discord in the work of Wiggers and Erlanger and his co-workers was brought about in some measure by Forward and Perme, who maintained that Erlanger, Gesell and Gasser tested largely the reaction of the peripheral arterioles in shock, whereas Wiggers determined changes in peripheral resistance due in part to alterations in the caliber of the capillaries. They held that they had direct proof that the early changes in the curves of arterial pressure in shock are associated with reduced peripheral resistance, but

that the arterioles at this time are constricted. They concluded that the point of vasodilation in shock is in the capillaries rather than in the arterioles. The fallacy of basing the presence of shock on readings of blood pressure alone, as pointed out by Porter and Cope, is again emphasized. The importance of blood volume diminution is again emphasized and offers an explanation for the development of shock not only in an animal that has bled, but also in one that has not.

#### SUMMARY

Most of the features of shock can be produced experimentally by bleeding an animal or allowing blood to escape into traumatized tissues. After hemorrhage and in shock increased blood nitrogen and decreased alkali reserve seem dependent on decreased volume flow of blood. Loss of plasma proteins, especially when associated with injury to the liver, is at present accorded prime importance in the development of secondary shock.

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## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—The death of Rudolf Kraus, director of the Instituto Bacteriologico de Chile, Santiago, at the age of 63 is announced. In 1897, Rudolf Kraus, then in Vienna where he did most of his scientific work, demonstrated that specific antibacterial serums produced precipitates in sterile filtrates of cultures of corresponding bacteria. He was the first secretary-general of the International Society for Microbiology.

In the department of pathology of the University of Chicago Paul R. Cannon has been promoted to professor and Oran I. Cutler has been appointed assistant professor.

William Hailock Park, director of research laboratories of the health department of New York, has been awarded the public welfare medal of the National Academy of Sciences for eminence in the application of science to public welfare.

Florence B. Seibert, member of the staff of the Sprague Institute for Medical Research and assistant professor of biochemistry in the department of pathology of the University of Chicago, has been appointed assistant professor of biochemistry in the Phipps Institute of the University of Pennsylvania.

William H. Howell has been elected chairman of the National Research Council for 1932-1933.

Stanhope Bayne-Jones has assumed the chairmanship of the division of medical sciences of the National Research Council for 1932-1933.

James L. Alloway has been appointed assistant professor of bacteriology in Cornell University Medical College.

In the University of Wisconsin Robert C. Bunts has been made assistant in pathology and Philip Levine instructor in bacteriology.

**American Journal of Parasitology.**—This journal, founded by Henry B. Ward, professor of zoology in the University of Illinois, under whose editorship eighteen volumes have been published, has been given by Professor Ward to the American Society of Parasitology. Henceforth the journal will be published as the official organ of the society, under the direction, for the next five years, of W. W. Cort, R. W. Hegner and F. M. Root, editorial committee.

**International Cancer Research Foundation.**—This foundation, which was organized on June 8, 1932, has been endowed with \$2,000,000 by William H. Donner, Villanova, Pennsylvania. The object of the foundation is to "increase interest in and the amount and quality of cancer research; develop new minds and theories; broaden the viewpoint of some investigators already in the field, and increase cooperation among scientists throughout the world, correlating results of their investigations and preventing duplication of work." The scientific advisory committee consists of James Ewing, New York; Burton T. Simpson, Buffalo, and Francis Carter Wood, New York.



# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

RENAL THRESHOLD FOR HEMOGLOBIN IN DOGS UNINFLUENCED BY MERCURY POISONING. W. H. HAVILL, J. A. LICHTY, JR., G. B. TAYLOR and G. H. WHIPPLE, J. Exper. Med. **55**:617, 1932.

The minimal, or depression, renal threshold for hemoglobin in the dog is not modified by moderate doses of mercuric chloride. This type of renal injury involves the epithelium of the convoluted tubules, but the glomeruli escape. We are unable to explain our findings if we assume that the tubular epithelium takes an active part in the passage of hemoglobin from the blood into the urine. The evidence points toward the glomerular tuft as responsible for its passage from the blood plasma into the tubules. The glomerular tuft establishes the true hemoglobin threshold under these conditions. If the convoluted tubules are normal, we note that hemoglobin is taken into the epithelium, and this explains the high initial renal threshold. With repeated injections of hemoglobin, this tubular epithelium becomes stuffed with hemoglobin pigment fractions and can absorb no more, which explains the minimal or depression threshold. Further injury of this tubular epithelium with mercury causes no change in this minimal renal threshold, unless we produce actual tubular obstruction.

AUTHORS' SUMMARY.

TOLERANCE FOR MERCURY POISONING INCREASED BY FREQUENT HEMOGLOBIN INJECTIONS. W. H. HAVILL, J. A. LICHTY, JR., and G. H. WHIPPLE, J. Exper. Med. **55**:627, 1932.

Frequent injections of superthreshold amounts of canine hemoglobin in dogs will cause deposits of pigment in the renal tubular epithelium. When this has happened, the dog will survive minimal lethal doses of mercuric chloride with little evidence of renal injury. In fact, some dogs will tolerate twice the minimal lethal dose without severe reaction. There is no evidence that continued injections of hemoglobin in these amounts will cause any injury or functional disability of the kidney. Rest periods will effect a disappearance of this pigment in the renal tubules. [See also articles by W. V. Newman and G. H. Whipple and by R. P. Bogniard and G. H. Whipple, J. Exper. Med. **55**:637 and 653, 1932.]

AUTHORS' SUMMARY.

EFFECTS OF PARATHORMONE AND AMMONIUM CHLORIDE ON THE BONES OF RABBITS. H. L. JAFFE, A. BODANSKY and J. E. BLAIR, J. Exper. Med. **55**:695, 1932.

A very large single dose of parathormone produced rapid and extensive decalcification of the bones of a young rabbit. Gradually increased doses of parathormone failed to produce significant changes in the bones of young and fully grown adult rabbits. Ammonium chloride, administered by stomach tube, was without effect on the bones, when given to rabbits treated with parathormone or to otherwise untreated rabbits. Calcium lactate did not promote the appearance of signs of parathormone overdosage in rabbits during treatment, nor were metastatic calcifications observed in the soft tissues after termination of the experiment. The rabbit is relatively very resistant to the decalcifying effects of gradually increased, repeated doses of parathormone, and therefore is not satisfactory material for experimental studies of the bone changes of chronic hyperparathyroidism. The

appearance of actively transforming bones of a young, growing rabbit, particularly at the sites of rapid growth, is not to be confused with that of the more severe fibrous processes produced by parathormone in guinea-pigs, dogs and rats.

## AUTHORS' SUMMARY.

EFFECT OF ANTITESTICULAR SERUM ON THE ENHANCEMENT VALUE OF TESTICLE EXTRACT. F. DURAN-REYNALS, *J. Exper. Med.* **55**:703, 1932.

The experiments reported here show that the infection-enhancing factor of testicle extract is neutralized in vitro by an antiserum against homologous testicle extract. An antiserum developed against a testicle extract of one species does not influence the enhancing and spreading factor of the extract from another species. Rabbits immunized against testicle extract do not exhibit any alteration in the spreading or enhancing effect of extracts employed later, even when the testicle extract used is from the same species as that employed for the immunization. Whether the in vitro inactivation of the active factor is a specific neutralization, or is the result of adsorption of the factor on the flocculate formed, has not been definitely determined. The fact that there is no neutralization in vivo, and that the antiserum acts only on extracts from the same species, when definite flocculation takes place, tends to emphasize the probability that the neutralization is not a direct one, but is incidental to the flocculation mentioned.

## AUTHOR'S SUMMARY.

THE RACES OF COMMON FIBROBLASTS. R. C. PARKER, *J. Exper. Med.* **55**:713, 1932.

The ability of fibroblasts to mature and to manifest their various potencies in any particular medium is inversely proportional to the growth energy which they exhibit in that medium. Fibroblasts having access to high concentrations of food substances in their environment do not mature, regardless of their origin or of the age of the animal from which they were derived. They behave as embryonic cells. Fibroblasts cultivated in vitro are potentially able to produce cells with the structural and functional properties commonly attributed to macrophages. This is true regardless of their origin or of the length of time that has elapsed since their isolation from the origin. The fibroblasts and the macrophage are considered to represent extreme functional and structural variations of the same cell type. The structural and functional characteristics displayed by fibroblasts in vitro vary according to their origin and according to the changes that take place in the composition of the medium in function of time.

## AUTHOR'S SUMMARY.

MOBILIZATION AND EXCRETION OF CALCIUM FOLLOWING OVERDOSAGE WITH IRRADIATED ERGOSTEROL. N. B. TAYLOR and C. B. WELD, *Brit. J. Exper. Path.* **13**:109, 1932.

The hypercalcemia following large doses of viosterol is due to withdrawal of calcium from the skeleton. Viosterol overdosage depresses the power of the intestine to excrete calcium. The calcium mobilized from the bones accumulates in the serum. A certain level of serum calcium having been reached, an increase in the urinary calcium follows. Viosterol in the amounts that produce hypercalcemia does not increase absorption of calcium from the intestine.

## AUTHORS' SUMMARY.

EFFECT OF VITAMIN DEFICIENCY ON THE STRUCTURE OF THE THYROID AND THYMUS GLANDS. A. W. SPENCE, *Brit. J. Exper. Path.* **13**:157, 1932.

An attempt was made to produce lymphadenoid goiter in twenty-four young rats by means of a diet consisting of: white flour, 82 per cent; olive oil, 8 per cent;

meat residue, 5 per cent; salt mixture (including an adequacy of potassium iodide), 5 per cent, and an unlimited supply of distilled water. The animals were on the diet for from nine weeks to thirteen months. Simple goiter was produced in fifteen of twenty rats examined, of which eight had slight goiters, five small goiters and two large ones. Lymphadenoid goiter did not arise. The production of simple goiter was independent of an absolute iodine deficiency. The factors concerned were dietetic insufficiency and infection. Outstanding was the occurrence of keratinization cysts in the thyroid gland in twenty-three of the twenty-four animals. These cysts arose from the vesicles, probably as a result of exhaustion. Similar cysts were found in the thymus glands of three animals on the diet, and are considered to be large Hassall's corpuscles and not an effect of vitamin deficiency. No support was found for the hypothesis that the cysts in the thyroid and thymus glands represent distentions of the thyroid-thymic lymphatics.

AUTHOR'S SUMMARY.

THE TOXICITY OF IRRADIATED ERGOSTEROL. J. B. DUGUID, M. M. DUGGAN and J. GOUGH, *J. Path. & Bact.* **35**:209, 1932.

Viosterol was found to be more toxic in rats fed on a certain synthetic vitamin-free diet of high calcium content than in rats on a normal diet. The absence of vitamins and the high calcium content were considered as possible determinants of the toxicity, and the high calcium content was found to be the more powerful. After reduction of the calcium and also of the phosphorus content of the diet, the evidence suggested that the absence of vitamins also contributed, but the evidence of this is not accepted as convincing. The toxicity was estimated chiefly on arterial and renal calcification. The reliability of these lesions as criteria is discussed. Arterial lesions are accepted as reliable indications of viosterol poisoning in the rat. Renal lesions were noted to occur in viosterol poisoning in two forms, namely, calcification of the renal arterioles, and calcareous casts in the renal tubules. The former appeared as part of a general arterial involvement and, as such, was taken as a reliable indication. The latter was found to be a lesion of too common occurrence in the rat, independently of the administration of viosterol, to be admitted as reliable evidence of toxicity.

AUTHORS' SUMMARY.

FOETAL RICKETS. J. P. MAXWELL, C. H. HU and H. M. TURNBULL, *J. Path. & Bact.* **35**:419, 1932.

The present case establishes beyond doubt the occurrence of rickets or of osteomalacia in the fetus. The relation of the two diseases is discussed in the light of recent investigations. It is concluded that both are metabolic, are doubtless due usually to food deficiencies and are probably both due to a deficiency of vitamin D. Their causal identity is not, however, proved. Although, therefore, the two cases bring additional evidence of the histologic identity of osteomalacia and rickets, they do not prove the identity of the two diseases.

AUTHORS' SUMMARY.

### Pathologic Anatomy

CONGENITAL MEDIAL SCLEROSIS OF THE CORONARY ARTERY. R. W. KISSANE and R. S. FIDLER, *Am. Heart J.* **7**:133, 1931.

The literature is reviewed, and a case is described. It concerns a noninflammatory primary thickening of the media of the larger coronary branches. The electrocardiogram shows a T-wave like that seen in the more familiar coronary sclerosis.

GONOCOCCUS ENDOCARDITIS. HENRY B. KIRKLAND, *Am. Heart. J.* 7:360, 1932.

A case of gonococcic endocarditis with autopsy is reported. Acute endocarditis followed urethritis and affected an aortic valve already damaged by rheumatism. In addition to acute vegetative endocarditis of the aortic valve, autopsy revealed chronic productive changes in both mitral and aortic valves, a subendocardial abscess with extension to the pericardium, diffuse suppurative and hemorrhagic pericarditis, acute intracapillary glomerular nephritis, acute focal interstitial nephritis, parenchymatous degeneration of the kidneys, infarction and suppuration of the spleen, degeneration and congestion of the liver, suppurative gastritis, pulmonary congestion, bilateral hydrothorax, ascites, conjunctival petechiae, suppurative leptomeningitis, gangrene of the legs and a decubitus ulcer.

AUTHOR'S SUMMARY.

AN UNUSUAL CASE OF LEFT-SIDED DISPLACEMENT OF THE HEART. LOUIS H. SIGLER, *Am. Heart J.* 7:388, 1932.

A case of sinistocardia, most likely congenital, is reported, which from perusal of the literature appears to be the only one of its kind on record. The outstanding features in the case were: 1. A healthy man, 50 years old, had gone through life without any circulatory or respiratory embarrassment. 2. The heart with its basal vessels was entirely displaced to the left of the spinal column. 3. Other mediastinal contents, such as the esophagus, trachea and bronchi, were likewise displaced. Although a history of injury was given, the injury seems to have been a mere coincidence, as there was no definite evidence of the presence of a pathologic condition that might be considered to be the cause of the displacement.

AUTHOR'S SUMMARY.

MONONUCLEAR ERYTHROPHAGOCYTOSIS IN THE BLOOD OF A NEW-BORN INFANT. A. F. ABT, *Am. J. Dis. Child.* 42:1364, 1931.

Abt briefly reviews the conditions in which mononuclear cells have been noted to phagocytose red blood cells. These conditions have invariably been pathologic and include paroxysmal hemoglobinuria, pernicious and secondary anemia, malarial infestation, sepsis and tuberculosis. This unusual phagocytic activity also occurs after splenectomy and most commonly is seen in endocarditis lenta. Abt observed this activity in an infant, aged 9 days, suffering from anemia of the new-born. Such erythrocyte-laden cells were observed for five days and then they disappeared. The phagocytic cells in this child were small macrophages, which seemed to have developed through the progressive hypertrophy of lymphocytes and monocytes.

GEORGE RUKSTINAT.

MIGRATORY PERITONITIS (SO-CALLED HEMATOGENOUS PERITONITIS) IN CHILDREN. S. A. WILE and O. SAPHIR, *Am. J. Dis. Child.* 43:610, 1932.

Twenty-four cases of peritonitis, twenty-one of which were diagnosed clinically as hematogenous peritonitis, proved at autopsy to be the result of an invasion of the peritoneal cavity by bacteria from primarily diseased intraperitoneal organs or structures close to the peritoneum, without perforation or without direct extension of the inflammatory process to the peritoneum. Sixteen of the twenty-four cases showed enteritis. The term "migratory peritonitis" seems appropriate for this type of case. Clinical reports of cases of hematogenous peritonitis without autopsies, or with autopsies but without complete histologic examination of the gastro-intestinal tract, such as comprise the bulk of the literature of so-called hematogenous peritonitis, should be discarded. Many of the cases in which the condition was reported as hematogenous peritonitis are examples of migratory peritonitis. The evidence supporting the occurrence of peritonitis directly following infection of the blood stream without an intermediary lesion

within or adjacent to the peritoneal cavity is inadequate. From our observations it is apparent that so-called hematogenous peritonitis may occur as part of a generalized metastatic pyemic process that results in abscess formation or infected thrombi within or adjacent to the peritoneal cavity. The ensuing peritonitis is the direct result of the abscess or thrombus. It is unlikely, however, that this form of peritonitis occurs in the absence of other metastatic pyemic phenomena.

AUTHORS' SUMMARY.

THE NORMAL HISTOLOGY OF INFANTS' BRAINS. HERMAN SCHWARZ, P. GOOLKER and JOSEPH H. GLOBUS, *Am. J. Dis. Child.* **43**:889, 1932.

Some well established, but not widely known, anatomic features characterizing certain stages in the developing brain are described. They often assume the form of circumscribed collections of deeply staining cells, which are often erroneously considered of inflammatory origin. Nine cases of intestinal intoxication are described. They revealed no evidence of an inflammatory or degenerative disease in the brain. Evidence is presented against a recent view that an encephalitic process in the diencephalon, affecting certain hypothalamic centers, causes the dehydration of intestinal intoxication. The conclusion is reached that no pathologic alterations were found in the brains studied to account for the clinical picture in intestinal intoxication.

AUTHORS' SUMMARY.

RHEUMATIC PNEUMONIA. B. A. GOULEY and J. EIMAN, *Am. J. M. Sc.* **183**:359, 1932.

Eight of nine cases of acute rheumatic fever showed an acute inflammation of the pulmonary tissue, with consolidation; the ninth showed pleurisy, with subacute pulmonary involvement. All were associated with acute rheumatic disease of the heart. The inflammatory pulmonary reaction consisted of an interstitial perivascular exudate of large endothelioid cells, morphologically identical with those found in rheumatic lesions of the heart and considered pathognomonic of rheumatic fever. Hemorrhage and fibrinous exudate were prominent features. We therefore consider that in many virulent cases a characteristic rheumatic pneumonia is to be found.

AUTHORS' SUMMARY.

DIABETIC GANGRENE. DAVID W. KRAMER, *Am. J. M. Sc.* **183**:503, 1932.

Various types of gangrene may occur in diabetes. The arteriosclerotic type and the so-called diabetic gangrene are the most common. Thrombo-angiitis obliterans and embolic gangrene are less so. The advisability of recognizing diabetic gangrene as a definite form is discussed. The fact that a certain number of diabetic persons with gangrene present clinical features and pathologic changes in the vessels, plus the fact that the gangrene may be of the moist variety, lends support in deciding that one is dealing with a form other than the well accepted arteriosclerotic type of gangrene. Analysis of 1,008 cases of diabetes mellitus observed between 1920 and 1930 shows that there were 58 cases of existing gangrene (5.75 per cent), 28 cases of threatened or impending gangrene and 89 cases of potential gangrene. Collectively, the three groups totaled 175 cases (17.3 per cent) of circulatory disturbances. If the more recent five year period (1926 to 1930) is considered, the incidence is higher: 6.78 per cent showed gangrene, and 19.75 per cent, deficient circulation in the extremities. My figures compare closely with the average statistics of others. Among 12,037 patients with diabetes, there were 756 cases of gangrene (a percentage of 6.28). Diabetic gangrene is the end-product of three main influences: the metabolic disturbances and their effect on the vessels and the tissues, changes in the arteries, with the resulting deficient blood supply to the parts, and infection.

AUTHOR'S SUMMARY.

THE PATHOGENESIS OF PERIGASTRIC ABSCESS COMPLICATING PEPTIC ULCER.  
HARRY A. SINGER and PETER A. ROSI, *Am. J. M. Sc.* **183**:600, 1932.

In twenty-nine of thirty cases of perigastric (including subphrenic) abscess, with origin in ulcer, convincing evidence was obtained of a previous acute perforation into the free abdominal cavity. The formation of the adhesions that are found about a perigastric abscess generally follows rather than precedes perforation. Between the time of recovery from the acute symptoms of perforation and the time of onset of manifestations of a perigastric abscess a period of fair health frequently intervenes. It is on this account that a patient often fails to associate the two illnesses. Unless a previous acute perforation is suspected and a minute-by-minute history of the initial symptoms obtained, information of the antecedent occurrence of a sudden, violent abdominal pain is often not elicited. In most instances, a perigastric abscess of ulcerative origin represents a neglected opportunity on the part of the patient, but perhaps more frequently on the part of the physician. Early recognition of perforated peptic ulcer, especially the type with mild postperforative symptoms, together with timely operation, will eliminate the greater number of perigastric abscesses.

AUTHORS' SUMMARY.

CHANGES IN POLYMORPHONUCLEAR LEUKOCYTES IN INFECTIONS. M. A. KUGEL and NATHAN ROSENTHAL, *Am. J. M. Sc.* **183**:657, 1932.

A study of the blood in various infections shows marked variation in the number of leukocytes and in the number of nonsegmented and segmented polymorphonuclear cells. In certain infections, the polymorphonuclear leukocytes show cytoplasmic granular changes. The changes include marked basophilia and, less frequently, vacuolization of the cytoplasm and appearance of certain cytoplasmic masses known as Döhle's bodies. The basophilic, or "toxic," granules are seen only in infections—most often in bacteremias. In streptococcic infections they occur late, particularly in subacute endocarditis; but in other conditions, especially those associated with more or less extensive suppuration (as pneumonia), they make their appearance early and usually persist for weeks after the crisis. "Toxic" granules occur in both segmented and nonsegmented polymorphonuclear neutrophils. The absence of "toxic" granules is suggestive of mild or localized infection. Persistence of this condition indicates a good prognosis or absence of complications. Absence of "toxic" granules or of degenerative changes in the cytoplasm of the polymorphonuclear leukocytes in cases of severe infection is unusual. The degenerative index offers a prognostic guide in infectious conditions: A decrease indicates recovery; a steady increase suggests either a complication or a bad prognosis; an index over 90 per cent is usually associated with severe infection, regardless of the clinical condition, and is unfavorable. Frequent observations of nuclear changes, such as segmentation and nonsegmentation, combined with consideration of cytoplasmic changes, is a most valuable aid in gaging the severity and the course of an infection.

FROM AUTHORS' SUMMARY.

ANATOMICAL CHANGES IN THE LIVERS OF DOGS FOLLOWING CONSTRICTION OF THE HEPATIC VEINS. J. P. SIMONDS and J. W. CALLAWAY, *Am. J. Path.* **8**:159, 1932.

The livers of dogs examined twenty-four, forty-eight and seventy-two hours and seven days after mechanical obstruction of the hepatic veins for periods of from seven to thirty minutes showed the following changes: a mean increase of 25 per cent in the liver weight-body weight ratio, due to edema and to swelling of the hepatic cells; swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two thirds of the hepatic lobules; marked dilatation of the perivascular lymphatic vessels surrounding the sublobular veins; the presence of hyaline thrombi in many central and sublobular veins;

intrasinusoidal cell masses of two types, i. e., small, compact, occluding masses probably originating in "conglutination thrombi" of red cells, and larger, more diffuse, branching cell masses; hemosiderosis of Kupffer cells.

AUTHORS' SUMMARY.

EXPERIMENTAL MUSCLE DEGENERATION. D. K. and H. R. FISHBACK, Am. J. Path. 8:193 and 211, 1932.

Degeneration of skeletal muscle has been produced by physical, chemical, bacterial, parasitic and pharmacologic trauma. The stages of degeneration produced were: slight granular clouding and swelling, with dimming of cross-striations; edema of fibers with prominent longitudinal fibrils; vacuolization; true granular degeneration, either albuminous or fatty; waxy degeneration with, further, lumpy disruption or granular disruption. The name "acute molecular degeneration of striated muscle" is suggested as a better descriptive and more inclusive term than "waxy degeneration."

Acute molecular degeneration of striated muscle in rabbits is a progressive process, including edema, fibrillar separation, vacuolization, hyaline change, lumpy disruption, granular change and finally complete dissolution of the muscle cytoplasm. This is associated with some cell exudation, with a high degree of phagocytic activity, and finally with repair. The course of repair is toward regeneration. The completeness of this process appears to depend on the destructiveness of the lesion, and not on the extent or the severity of the degeneration of muscle fibers. If the destruction of the sarcolemma is not too severe, and if the stroma remains, these, with surviving muscle nuclei, form the integral factors for muscle restoration. With diffuse destruction of tissue, scarring results.

AUTHORS' SUMMARIES.

VITAL STAINING OF THE RABBIT'S AORTA IN THE STUDY OF ARTERIOSCLEROSIS. G. L. DUFF, Am. J. Path. 8:219, 1932.

In rabbits, intravenous injection of a suitable quantity of a solution of trypan blue results in well marked staining of the wall of the aorta within sixteen hours. The depth of color as seen on the intimal surface is not uniform, some areas being more deeply stained than others. The differences in intensity of staining become more prominent with increase in the length of the experiment. The variations are the result of irregularities in the staining of the outer layers—the adventitia and the outer portion of the media. The deeply staining areas correspond to the areas in which the aortic wall is most plentifully supplied by vasa vasorum, while the pale staining areas correspond to those in which the vascularization of the aorta is least abundant. The staining of the wall of the aorta is chiefly due to the escape of the dye through the capillary endothelium, which is much more permeable to trypan blue than is the lining endothelium of the aorta. The local variations in depth of staining in the aorta are thus dependent on the degree of vascularization of its walls. The production of an inflammatory reaction in the external layers of the aorta brings about a local increase in capillary permeability to trypan blue and as a result a stronger staining of the wall in the inflamed area.

AUTHOR'S SUMMARY.

THE QUESTION OF A SPECIFIC MYOCARDIAL LESION IN HYPERTHYROIDISM. W. LEWIS, Am. J. Path. 8:255, 1932.

In some cases of hyperthyroidism, certain degenerative and inflammatory changes that occur in the myocardium indicate a toxic origin and suggest a toxin circulating in the blood stream. Two cases showing such myocardial changes are reported.

AUTHOR'S SUMMARY.

ATELECTASIS AS A FACTOR IN THE EVOLUTION OF CHRONIC FIBROID PULMONARY TUBERCULOSIS. HERMAN HENNEL, *Am. Rev. Tuberc.* **23**:461, 1931.

In so-called "advanced unilateral fibroid phthisis," massive atelectasis of large portions of pulmonary tissue, occasionally involving most of one lung, is probably the essential mechanism. The tuberculous process in the lung may be minimal, and its clinical significance may become relatively unimportant. The marked pulmonary fibrosis, which rapidly develops after atelectasis occurs, may submerge the tuberculous process, and the bronchiectasis that follows in the wake of the pulmonary fibrosis may determine the clinical features and course. In the evolution of fibroid phthisis of lesser degree, atelectasis involving smaller or larger areas of pulmonary tissues is probably an important factor. It is conservative, and may be responsible for the mild clinical course as well as the slow progress of the pathologic process after it is established.

H. J. CORPER.

ATELECTASIS IN PULMONARY TUBERCULOSIS. E. KOROL, *Am. Rev. Tuberc.* **23**:493, 1931.

Atelectasis is common in tuberculosis and is responsible for contraction of the chest and lung, limited mobility, feeble or absent vesicular murmur and decreased complemental space. The small lung with a displaced mediastinum and diaphragm frequently develops in spite of the absence of fibrosis and adhesions; it is due to a diminished amount of air in the lung and a fall in intrapleural pressure. The pendulum movement of the mediastinum observed in bronchostenosis and atelectasis occurs also in tuberculosis with preponderantly unilateral distribution. Paradoxical breathing accompanies tuberculous disease of the lung; it is believed to be the cause of the characteristic râles. In many cases, this breathing from one lung into the other spreads the tuberculous infection.

H. J. CORPER.

PULMONARY ASBESTOSIS. K. M. LYNCH and W. A. SMITH, *Am. Rev. Tuberc.* **23**:643, 1931.

Lynch and Smith have collected reports of 172 cases of pulmonary asbestosis. In 27, the diagnosis was confirmed by the finding of asbestosis bodies in the sputum or in the lung juice obtained through puncture or by necropsy. Necropsy was made in 18 cases. In 3 of these, the disease was complicated by pulmonary tuberculosis; in 3, by lobar pneumonia, and in 3, by bronchopneumonia. One case was terminated by a traumatic death. In 4, a complete report was lacking, but the statement was made that necropsy confirmed the diagnosis. Including the very first report of a case, that of Murray in the *Charing Cross Gazette* of 1900, which apparently received little attention until it was resurrected by Cooke, there are now 4 records of necropsy on uncomplicated pulmonary asbestosis.

H. J. CORPER.

CHOLESTEROL-THORAX IN TUBERCULOSIS (CHOLESTEROL PLEURISY). HARRY M. STEIN, *Arch. Int. Med.* **49**:421, 1932.

The case reported is one of cholesterol pleurisy occurring in a man who had a history of pleural effusion following pneumonia. In the absence of a clearer history it might be that the condition was originally one of tuberculous pleurisy with effusion and not of pneumonia. The patient had tuberculosis involving the central nervous system many years after his probable tuberculous pleurisy, and there was postmortem evidence of tuberculosis of the pleurae. Cholesterol-thorax might be looked on, in the majority of instances, as an unusual end-result of a long-standing pleural effusion that was originally tuberculous. One might assume that, in view of the fact that the majority of primary pleural effusions are tuberculous in origin, cholesterol-thorax simply means that an old tuberculous effusion



which has not been cured and which has not caused death will, in some instances, eventually become cholesterolized. Cholesterol metabolism is, of course, as yet an unsolved problem, and it may be that an old pleurisy becomes cholesterolized only in a person who has an abnormal cholesterol chemistry, this observation, of course, being theoretical. Reports vary as to the cholesterol chemistry in pulmonary tuberculosis; it would seem that the cholesterol content in the blood in tuberculous infections may be increased or decreased. Eichelberger and McCluskey claimed that a rising or stationary hypercholesteremia indicates immunity and resistance, while a falling or low cholesterol content of the blood indicates a lowering in immunity and resistance.

AUTHOR'S SUMMARY.

THE STUDY OF CONCRETIONS BY X-RAYS. K. H. BAUER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:299, 1931.

Roentgen examination of concretions, inside and outside of the body, is simple and reliable and gives almost as much information as study of microscopic sections. Since the different substances composing the stones have entirely different densities, a diagnosis of the chemical structure can be obtained in most instances from x-ray pictures, before removal of the calculi from the body. In 11.6 per cent of 250 patients with multiple gallstones, fragmentation of concretions, leading to new formation of stones, could be demonstrated with this method. The intact stones showed clefts as the first stage of fragmentation. The physical process of fragmentation is explained on the basis of the view of Goldschmidt.

C. ALEXANDER HELLWIG.

CHRONIC PASSIVE CONGESTION OF THE SPLEEN. E. JAEGER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:334, 1931.

Chronic passive congestion of the spleen was produced in dogs, and the morphologic changes in the spleen were studied. Continuous congestion caused induration characterized by marked thickening of the trabeculae and of the capsule, dilatation of the trabecular veins and atrophy of the malpighian bodies. Remittent congestion produced hyperplasia and dilatation of the sinus, thickening of the reticulum and moderate fibrosis of the pulp and malpighian corpuscles. Furthermore, the author observed siderofibrosis, so-called Gandy-Gamna's nodules, in the wall of larger blood vessels of the trabeculae and in the capsule. Jaeger concludes that splenic tumor accompanying cirrhosis of the liver may be due to passive congestion alone, and he rejects the opinion of many writers that splenomegaly in cirrhosis of the liver is due to a toxic agent influencing simultaneously the spleen and the liver.

C. ALEXANDER HELLWIG.

PANCREATIC FERMENTS IN THE BILE OF THE GALLBLADDER. A. WERTHEMANN, *Verhandl. d. deutsch. path. Gesellsch.* **26**:343, 1931.

The average content of amylase in the bile of fifty-one gallbladders was 312 (Wohlgemuth's method). Amylase is apparently not secreted by the mucosa of the gallbladder, since it is practically absent in cases of obstruction of the cystic duct. When there is a communication between the pancreatic and the common bile duct, pancreatic juice may enter into the bile ducts. This, however, does not by itself cause bilious peritonitis without perforation, because in such cases, it was found, the amylase content of the bile is much lower than that in the pancreatic juice. Also the bilious imbibition of the surrounding tissue of the gallbladder, as found at autopsies, is without relation to the amylase content of the bile, but depends on chronic inflammatory fibrosis of the wall of the gallbladder and on the viscosity of the bile.

C. ALEXANDER HELLWIG.

LARGE GROUND PREPARATIONS OF THE VERTEBRAL COLUMN. L. MICHAELIS, *Verhandl. d. deutsch. path. Gesellsch.* **26**:363, 1931.

The principle of Christeller's large sections of parenchymal organs is applied to the study of lesions of the bones. Portions of the vertebral column, consisting of from four to eight vertebrae, the half of the femur and other long bones are sawed longitudinally into halves and one of them is ground and polished. The thin preparations can be studied microscopically by reflected light. Photomicrographs are demonstrated which were made from these sections, with a magnification of 1,000 diameters. This new method permits the histotopographic study of lesions of the bones and is of great practical value.

C. ALEXANDER HELLWIG.

THE ARCHITECTURE OF THE ASCENDING AORTA AND ITS PATHOLOGIC SIGNIFICANCE. F. ORSÓS, *Verhandl. d. deutsch. path. Gesellsch.* **26**:365, 1931.

The study is based on 15,000 serial sections from 200 aortae. A system of muscular-elastic bundles is described that shows a typical arrangement in the wall of the ascending aorta. The longitudinal bundles of this system and the margins of the sinus are predisposed to atherosclerosis. It is in these structures that histologic examination often reveals definite atherosclerotic changes, while macroscopically the aorta still appears completely normal.

C. ALEXANDER HELLWIG.

PATHOLOGIC ANATOMY OF THE SALIVARY GLANDS. E. KIRCH, *Verhandl. d. deutsch. path. Gesellsch.* **26**:387, 1931.

For the first time, invasion of the excretory ducts of salivary glands by pyogenic cocci has been demonstrated in cases of septicemia and pyemia. Often small hyaline or white thrombi were found in blood vessels of the glands, but never abscesses in the glandular parenchyma. Arteriosclerosis is a frequent finding in salivary glands and accompanies most cases of arterial hypertension. Atherosclerosis of the larger arteries, however, is uncommon. Glycogen was found in all salivary glands in cases of sudden death and seems to be normal in these glands. Brown pigmentation of the epithelial cells of the intercalated ducts is common in older people.

C. ALEXANDER HELLWIG.

PHYSIOLOGIC TRANSFORMATION OF GASTRIC GLANDS. H. HAMPERL, *Verhandl. d. deutsch. path. Gesellsch.* **26**:392, 1931.

In 400 organs fixed immediately after death, the structure of the fundic glands was studied with special staining methods (methylviolet and Best's carmine stain). The zymogenic cells can slowly disappear during the physiologic process of replacement of shed cells and can be replaced by mucous neck cells, until finally the whole fundic gland consists of goblet cells. The glands found after this physiologic process cannot be distinguished from the pseudopyloric glands due to regeneration of defects of the mucosa.

C. ALEXANDER HELLWIG.

### Microbiology and Parasitology

THE EARLY CELLULAR REACTIONS IN THE LUNGS OF RABBITS INJECTED INTRAVENOUSLY WITH HUMAN TUBERCLE BACILLI. ARTHUR J. VORWALD, *Am. Rev. Tuberc.* **25**:74, 1932.

The cells that respond first to an intravenous injection of tubercle bacilli of the human type into rabbits (0.1 mg. per kilogram of animal weight) are the polymorphonuclear leukocytes. This reaction, while transitory, is not one of negligible significance. It is through the agency of the polymorphonuclear leukocytes that the site of subsequent development of the tubercle is determined. These

cells phagocytose the tubercle bacilli in the blood stream and stagnate in small accumulations in the capillaries where the current is slow. In this way, injected bacilli are concentrated and localized. The focal accumulations of polymorphonuclear leukocytes form the groundwork from which the epithelioid tubercles develop. Mononuclear exudate cells are preponderant in the cellular response after the first few hours (from fourteen to eighteen hours on). They mingle with the polymorphonuclear leukocytes, phagocytose and digest them and take up their content of tubercle bacilli. In this way, by replacement of the leukocytic mass, the mononuclear cell tubercle, ultimately the epithelioid tubercle, develops. The increase in mononuclear exudate cells in the field of inflammation, in almost total absence of mitotic figures in such foci, indicates that the majority of reacting cells of this type are hematogenous in origin.

APPENDICEAL OXYURIASIS. H. GORDON, *Ann. Int. Med.* **4**:1521, 1931.

Gordon investigated the occurrence of oxyurids in 20,969 appendixes that had been received for routine diagnosis at the pathologic laboratories of the University of Michigan from 1894 to 1930. Only surgical specimens were considered. Oxyurids were found in 221 appendixes (1.05 per cent). Infestation was found to be much more common during the first decade of life and almost entirely absent after the forty-sixth year. There was no significant seasonal predilection for the occurrence of oxyuriasis. The significantly greater incidence of appendical oxyuriasis during the past decade is probably related to the increased consumption of uncooked leafy vegetables and raw fruits.

WALTER M. SIMPSON.

EXPERIMENTAL AGRANULOCYTOSIS (*SALMONELLA SUIPESTIFER*). B. M. FRIED and WILLIAM DAMESHER, *Arch. Int. Med.* **49**:94, 1932.

The purpose of the present study has been to determine the possible similarity between the blood picture as seen in agranulocytosis in man and that found in a form of experimental sepsis in rabbits. The results have shown that there are close similarities between the agranulocytosis resulting from the hematogenous infection of rabbits with *Salmonella suipestifer* and that observed in cases of agranulocytic angina in man. Thus, the reaction in severe cases of human agranulocytosis corresponds to that of the animals that received overwhelming doses of bacteria, i. e., persistent neutropenia and intense necrosis of the bone marrow without signs of regeneration. A close similarity likewise exists between the "recovery phase" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria, i. e., marked histiomonocytosis. Incidentally, Schilling's clinical concept of "regenerative" and "degenerative" types of polymorphonuclear "shift" was confirmed by these experiments.

AUTHORS' SUMMARY.

THE EFFECT OF SODIUM RICINOLEATE ON THE GONOCOCCUS. C. PHILLIP MILLER, JR., and R. CASTLES, *J. Bact.* **22**:339, 1931.

Sodium ricinoleate does not "detoxify" gonococci but rather increases their lethal action for mice. It does not destroy the antigenic properties of gonococci.

AUTHORS' SUMMARY.

COLONIES OF HEMOLYTIC STREPTOCOCCI IN SCARLET FEVER. R. TUNNICLIFF, *J. Infect. Dis.* **49**:357, 1931.

During the course of scarlet fever, especially during convalescence and in complications, atypical hemolytic streptococci were isolated alone or associated with typical *S. scarlatinae*. They appeared to have dissociated from the typical, or "normal," slightly granular, conical scarlatinal colonies and differed from them

by forming on heated blood agar granular or smooth convex colonies and rough conical colonies with regular edges. The slightly granular and the rough conical colonies did not change chocolate agar; the granular convex colonies turned it green, while the smooth convex colonies varied in this respect. By the opsonic test, the cocci from dissociated colonies were not opsonified like the group of streptococci specific for scarlet fever, but by subculturing they were reverted, both colonially and immunologically, to typical scarlatinal streptococci. AUTHOR'S SUMMARY.

THE EFFECT OF HEAT, STORAGE AND CHLORINATION ON THE TOXICITY OF STAPHYLOCOCCUS FILTRATES. E. O. JORDAN, G. M. DACK and O. WOOLPERT, *J. Prev. Med.* 5:383, 1931.

The toxic substance present in staphylococcus filtrates causing gastro-intestinal derangement is not completely destroyed by exposure for thirty minutes to the temperature of boiling water. Some diminution in toxic power may, however, possibly be caused by heating, even at temperatures below 100 C. Similarly, the toxic quality does not disappear after storage at a low temperature for as long as sixty-seven days, but is perhaps somewhat weakened. Contact for three minutes with a rather strong dose of chlorine did not destroy the toxic quality.

AUTHORS' SUMMARY.

A CASE OF FOOD POISONING APPARENTLY DUE TO STAPHYLOCOCCUS. E. O. JORDAN and J. R. HALL, *J. Prev. Med.* 5:387, 1931.

An outbreak of food poisoning from eating chicken gravy was apparently caused by the products of growth of a staphylococcus, which in pure culture formed substances that, when swallowed by human volunteers, reproduced the typical picture of the original outbreak.

AUTHORS' SUMMARY.

GLANDULAR FEVER—THE PROTOZOAL NATURE OF THE EXPERIMENTAL DISEASE. J. O. W. BLAND, *Brit. J. Exper. Path.* 12:311, 1931.

The disease of rabbits, previously called "experimental glandular fever," which followed the inoculation of blood from two patients with glandular fever is caused by a protozoan of the genus *Toxoplasma*. This protozoan closely resembles *T. cuniculi* and is immunologically identical with it. The disease it causes is indistinguishable from that produced by *T. cuniculi*. The protozoan differs from ordinary *T. cuniculi* in its greater virulence for rabbits and in its power to infect monkeys, in which it produces a disease much like human glandular fever. No similar disease has been produced in rabbits of the same stock by control inoculations with normal rabbit or normal human blood or with blood from febrile people. The evidence suggests that human glandular fever may be caused by the protozoan described, but this requires confirmation.

AUTHOR'S SUMMARY.

THE RESISTANCE TO HEAT AND DISINFECTANTS OF PROTEIN-FREE ELUATES OF A BACTERIOPHAGE AND FOWL-POX VIRUS. I. J. KLIGLER and L. OLITZKI, *Brit. J. Exper. Path.* 12:393, 1931.

Data are presented showing that the effect of heat and, more particularly, that of various disinfectants on a bacteriophage and fowl-pox virus, is weakened by the presence of protein. There is a general similarity in the reaction of the pure fowl-pox virus and bacteriophage to disinfectants, but there are differences in their relative susceptibility to one or another antiseptic. Both pure virus and pure bacteriophage are less sensitive to the action of phenol and ether than to any of the other reagents used.

AUTHORS' SUMMARY.

HEMATOGENOUS TUBERCULOSIS OF THE TONSILS. C. KRAUSPE, *Verhandl. d. deutsch. path. Gesellsch.* **26**:278, 1931.

Except in the first stages, it cannot be decided from histologic examination, whether in a given case tuberculosis of the tonsils is due to hematogenous or to oral infection. The problem was studied on rabbits after oral and intravenous injection of bovine tubercle bacilli. In both instances, the tubercles were found in the lymphoid tissue of the tonsils, especially in the area near the lymph follicles. In children with tonsillar tuberculosis, hematogenous tubercles are frequently found in the bones of the base of the skull. This fact suggests that tuberculosis of the tonsils also is most often due to a hematogenous infection.

C. ALEXANDER HELLWIG.

EXPERIMENTS ON ACUTE LYMPHOCYTOSIS. G. WALLBACH, *Verhandl. d. deutsch. path. Gesellsch.* **26**:352, 1931.

Suspensions of proteus and colon bacilli were injected intravenously into rabbits. In a few minutes, marked leukopenia with relative lymphocytosis was found, which continued for the most part until the next day. In this stage, death occurred in several of the animals. The following day, sometimes earlier, the condition of the other rabbits improved, at which time increasing leukocytosis was demonstrated. When the injection of bacteria was repeated, leukopenia was less, noticeable; after the third injection it was almost completely missed. Leukocytosis is, according to the author, evidence of active immunization and follows an initial stage of destruction of granulocytes.

C. ALEXANDER HELLWIG.

COMPARATIVE PATHOLOGY OF PARASITIC DISEASES OF THE BRAIN. L. SINGER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:357, 1931.

Six cases of coenurosis were studied histologically. The larval stage of *Taenia coenurus* causes typical mesenchymal changes in the brain substance, which resembles the tissue reaction in echinococcic disease of the liver. The parasite was found lying in a necrotic area, surrounded by epithelioid cells in pallisade arrangement and giant cells. At the periphery, a wall of lymphocytes had formed with typical lymph follicles.

C. ALEXANDER HELLWIG.

THE CULTIVATION OF SPIROCHAETA PALLIDA. R. R. HÖLTZER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:320, 1931.

The so-called "cotton" culture mediums of Wrublewski, consisting in the use of cotton instead of animal tissues for adsorption of oxygen, proved unsatisfactory for growing *Spirochaeta pallida*. However, addition of 10 per cent rabbit or sheep serum gave a luxuriant growth, equal to the one obtained in broth containing pieces of liver. A layer of petrolatum on the surface to exclude oxygen was necessary for the growth.

I. DAVIDSOHN.

CHEMICAL CHANGES IN THE SERUM OF GUINEA-PIGS WITH EXPERIMENTAL TUBERCULOSIS. T. WOHLFEIL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:387, 1931.

The tryptophane was elevated in direct proportion to the severity of the disease. The increase varied from 21 per cent in animals with only local glandular involvement to 94 per cent in animals with diffuse glandular and visceral changes. A number of animals infected with a strain of low virulence showed, on superinfection with a very virulent strain two months later, markedly increased resistance and considerable rise in serum tryptophane as compared with animals that did not present increased resistance to superinfection. Tyrosine, nonprotein nitrogen and aminonitrogen were within normal range.

I. DAVIDSOHN.

HASTENING EXPERIMENTAL TUBERCULOSIS IN THE GUINEA-PIG. ADOLF HOCHWALD and ERICH TAUB, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72:462**, 1931.

The injection of virulent tubercle bacilli was followed in twenty-four hours with a series of ten daily injections of a typhoid vaccine. The specific tuberculous changes were farther advanced and more diffusely spread out in animals treated with the vaccine than in controls. The apparent hastening of the tuberculous process is brought about, in the author's opinion, by the nonspecific effect of the foreign protein and the specific action of the typhoid toxin on the reticulo-endothelial system, and to some extent also by the loss of appetite, the fever and the peritoneal irritation.

I. DAVIDSOHN.

TUBERCULOSIS OF THE BRONCHIAL LYMPH NODES IN ADULTS. T. HAUSMANN, *Ztschr. f. Tuberk.* **61:218**, 1931.

In contradistinction to the usual teaching it is claimed that tuberculosis of the lymph nodes in adults is not rare. It occurs rarely in the presence of progressive pulmonary tuberculosis, but frequently in the absence of pulmonary lesions. The disease is diagnosable essentially by percussion.

MAX PINNER.

MONOCYTOSIS IN PULMONARY TUBERCULOSIS. H. VOS, *Ztschr. f. Tuberk.* **61:305**, 1931.

Monocytosis is frequently found in tuberculosis of adults, more frequently when the disease is severe than when it is slight. In favorable cases, monocytosis is more frequently associated with lymphocytosis; in unfavorable cases, with lymphopenia and shifting to the left. According to Schilling, monocytosis in tuberculosis is in most cases not to be considered as a prognostically favorable sign. The appearance of monocytosis is prognostically ambivalent.

MAX PINNER.

THE DIAGNOSTIC SIGNIFICANCE OF FECAL EXAMINATION FOR TUBERCLE BACILLI. D. SZÜLE, *Ztschr. f. Tuberk.* **61:422**, 1931.

In intestinal tuberculosis, the bacilli are demonstrable in every case. In pulmonary tuberculosis, they are demonstrable only in open cases. Frequently bacilli are demonstrable in the stools when they are not demonstrable in the sputum.

MAX PINNER.

TUBERCLE BACILLI IN THE BLOOD OF TUBERCULOUS PATIENTS. M. HUTTIG, *Ztschr. f. Tuberk.* **62:35**, 1931.

Only seven of fifty-nine patients with pulmonary tuberculosis, in most of them far advanced, gave blood cultures positive for tubercle bacilli according to Löwenstein's method.

MAX PINNER.

FURTHER STUDIES OF THE DISASTER IN LÜBECK. B. LANGE, *Ztschr. f. Tuberk.* **62:335**, 1931.

At the time when the first death occurred following the BCG vaccination in Lübeck, a strain marked BCG 143 was present in the Lübeck laboratory. Careful tests for virulence made it quite certain that this strain was not one of BCG but a strain of a human tubercle bacillus of very low virulence for guinea-pigs. So far all evidence points to the assumption that pure BCG is unlikely to undergo sudden increases in virulence. Statements to the contrary are based on experimental work which so far is lacking confirmation. Although other

workers were able to derive morphologically different colonies from BCG, Petroff's contention that the S form is virulent has not been confirmed. It is concluded again that disease and death were caused in the vaccinated children because a pathogenic tubercle bacillus other than BCG had been administered. Since in vaccines a tubercle bacillus of low virulence was demonstrated, and since a tubercle bacillus of similar behavior was isolated from the diseased children this statement seems well founded, especially since atypical strains of such low and labile virulence for guinea-pigs are quite rare.

MAX PINNER.

**TUBERCULOSIS IN THE FIRST THREE YEARS OF LIFE.** J. OSTENFELD and R. KJER-PETERSEN, *Ztschr. f. Tuberk.* **62**:369, 1931.

A total of 260 families in each of which at least one member had proved open tuberculosis, and which had been observed during the years from 1919 to 1925, were studied in regard to the fate of children born in these families. A total of 245 children were available for the study. Of these 245 children, 19, or 7.8 per cent, died. In 12, the cause of death was undoubtedly tuberculosis. This percentage is far below the 25 or 30 per cent which is claimed by Calmette. From 1908 to 1925, 295 children were known to the dispensaries in Copenhagen to have had a positive Pirquet test within the first three years of life. In 1930, 293 of these children could be traced. Of 53 children who had a positive reaction during the first year of life, 33 were alive after at least five years. Of 91 children with a positive Pirquet test in the second year of life, 77 were living, and of 149 children with a positive test in the third year of life, 134 were living. Of all children who had died, only 6 had died after the age of 5 and only one of these had died of tuberculosis. It is concluded that a positive Pirquet test even in the first two years of life does not necessarily indicate active disease.

MAX PINNER.

## Immunology

**IMMUNITY TO VACCINATION BY INJECTION OF TESTICULAR VACCINE VIRUS.** S. P. KRAMER, *J. Infect. Dis.* **50**:119, 1932.

When rabbits were treated by repeated injections of testicular vaccine virus that had been filtered through a basic filter, they became immune to vaccinia. When virus of dermal origin was used, immunity did not follow.

AUTHOR'S SUMMARY.

**EFFECT OF TYPHOID FEVER AND PREVIOUS TYPHOID VACCINATION ON ANTIBODY RESPONSE TO TYPHOID VACCINE.** R. F. FEEMSTER, *J. Infect. Dis.* **50**:121, 1932.

Persons who have had typhoid fever or who have been inoculated with typhoid vaccine produce larger amounts of antibodies in response to subsequent inoculations with typhoid vaccine than those who have not had typhoid or have not been vaccinated against the disease. The routine injection of three doses of typhoid vaccine may not give as high a degree of immunity as has been believed. A second or third injection some weeks or months after the first would seem to be indicated. The Widal reaction is less reliable in the case of a person who has been vaccinated two or more times than in that of a person who has been vaccinated only once. The complement-fixation reaction is no more reliable than the agglutination reaction in diagnosing infection with the typhoid bacillus. The difficulties and uncertainties encountered in carrying out and interpreting bactericidal tests render the procedure unsatisfactory for the routine diagnosis of typhoid fever. The severity of the reaction to the injection of typhoid vaccine is in no way related to the immunity that is formed in response to the injection.

AUTHOR'S SUMMARY.

STAPHYLOCOCCUS AUREUS AGGLUTININS IN TUBERCULOUS EFFUSIONS. M. PINNER and M. VOLDRICH, *J. Infect. Dis.* **50**:143, 1932.

Tuberculous effusions invariably contain a factor that behaves serologically like an agglutinin specific for *Staphylococcus aureus*. The majority of nontuberculous effusions tested do not agglutinate *S. aureus*; neither does blood serum from tuberculous patients.

AUTHORS' SUMMARY.

PRECIPITINOGENIC ACTION OF MINUTE QUANTITIES OF OVALBUMIN. L. HEKTOEN and A. G. COLE, *J. Infect. Dis.* **50**:171, 1932.

Two rabbits were immunized against ovalbumin by injections of 0.000,047 Gm. and 0.000,27 Gm., respectively, of protein nitrogen, corresponding to approximately 0.000,29 Gm. and 0.001,7 Gm. of ovalbumin. The antisera obtained gave precipitates with the homologous antigen in the dilution 1:100,000.

AUTHORS' SUMMARY.

ANTIGENIC PROPERTIES OF CARBOHYDRATE AND PROTEIN FRACTIONS OF MENINGOCOCCI. J. ZOZAYA and J. E. WOOD, *J. Infect. Dis.* **50**:177, 1932.

The polysaccharide and the "nucleoprotein" obtained from meningococci of the different types, gonococci and *Micrococcus catarrhalis* have similar immunologic properties and are not type-specific. The carbohydrate-precipitable substance in immune serums appears late (fourth month or more) in the immunization of animals. There is no parallelism between the agglutination test and the polysaccharide precipitin test.

AUTHORS' SUMMARY.

NATURAL IMMUNITY TO STAPHYLOCOCCAL TOXIN. L. M. BRYCE and F. M. BURNET, *J. Path. & Bact.* **35**:183, 1932.

The distribution of staphylococcic antitoxin in human serum according to age is similar to that obtaining for diphtheritic antitoxin. In about 50 per cent of infants, the blood at birth contains the same concentration of antitoxin as the mother's blood, but gross divergences are not uncommon and point to a considerable lag in the passage of antitoxin across the placenta. Natural staphylococcic antitoxin is frequently present in domestic rats, and evidence is presented which indicates that its occurrence is a true antibody response to contact with the specific antigen. In many persons, the Dick type of reaction to intradermal injection of staphylococcic filtrates is not a manifestation of the activity of staphylococcic toxin.

AUTHORS' SUMMARY.

CELLULAR IMMUNITY TO SNAKE VENOM AND STAPHYLOCOCCAL TOXIN. F. M. BURNET, C. H. KELLAWAY and F. E. WILLIAMS, *J. Path. & Bact.* **35**:193 and 199, 1932.

The perfused heart muscle of a rabbit immunized to cobra venom was less susceptible to the action of this venom than that of a normal rabbit. Adding small amounts of immune serum to the bath in which the normal isolated auricle was suspended caused it to behave with venom like the auricle from an immunized animal, suggesting that in the immunized animal there might be an accumulation of antibody in the tissue spaces not readily removable by perfusion. In immunization of animals with staphylococcic toxin it was found that there is an accumulation of antibody in the tissue spaces which cannot be removed by perfusion. The results throw doubt on the value of the perfusion experiment for the demonstration of antitoxic cellular immunity.



IMMUNE SERA AGAINST FOWL-TUMOUR VIRUSES. C. H. ANDREWES, J. Path. & Bact. **35**:243, 1932.

The development of antibodies to the Rous virus may be followed in the serums of fowls bearing either of two slow-growing fibrosarcomas (MH1 and CT10). Potent antiserums were not found in fowls that had borne tumors for less than five months. Neutralization of Rous virus by immune serum is more complete when the mixtures are incubated at 37 C. It is possible to recover virus from an inactive serum-virus mixture, provided that serum and virus have not been long in contact. Resemblances between the serums under discussion and other antiviral serums are pointed out.

AUTHOR'S SUMMARY.

STAPHYLOCOCCAL TOXIN AND ANTITOXIN. H. J. PARISH and W. H. M. CLARK, J. Path. & Bact. **35**:251, 1932.

Staphylococcic septicemia is comparatively rare and rapidly fatal. Staphylococcic toxin can be prepared in an atmosphere containing 25 per cent carbon dioxide in Parker's 4 per cent peptone buffered broth, on Burnet's 0.8 per cent agar plates, and in Winchester bottles containing 0.8 per cent agar to which Parker's broth is added after forty-eight hours' growth. The toxin kills rabbits, guinea-pigs and mice when given intravenously, produces cutaneous lesions in rabbits and guinea-pigs, and is actively hemolytic. It is rapidly converted into toxoid by formaldehyde. Horse antitoxin can be prepared that protects rabbits against a lethal dose of toxin injected intravenously. It is best titrated by injecting mixtures of toxin and antitoxin intracutaneously into guinea-pigs, and by testing its antihemolytic power. The antitoxin can be concentrated by ammonium sulphate precipitation. When given intravenously to rabbits prior to a certainly fatal dose of virulent living culture, it prolongs and in some cases saves life. The favorable results that have attended its use in a small number of patients justify more extensive clinical trial.

AUTHORS' SUMMARY.

THE EFFECT OF LEAD ON THE PRODUCTION OF IMMUNE SUBSTANCES. F. W. BICKERT, Arch. f. Hyg. **106**:271, 1931.

Rabbits given a series of daily subcutaneous injections of 1 cc. of a 5 per cent solution of lead acetate appear to produce a greater yield of antisheep hemolysin than rabbits not so treated. The maximum titer is reached more slowly in the lead-treated animals than in the controls. Preliminary injections of lead salts appear to favor production of agglutinin and antitoxin, also, but to inhibit the production of precipitin.

ARTHUR LOCKE.

THE EFFECT OF BENZENE AND TURPENTINE ON THE PRODUCTION OF IMMUNE SUBSTANCES. F. W. BICKERT, Arch. f. Hyg. **107**:1, 1931.

Rabbits given intravenous injections of washed sheep erythrocytes produce a diminished and retarded yield of antisheep hemolysin when they have previously received subcutaneous injections of benzene. Subcutaneous injections of turpentine appear to favor the production of hemolysin and leukocytes.

ARTHUR LOCKE.

THE LIPOLYTIC CONTENT IN SPUTUM IN PULMONARY TUBERCULOSIS. S. PUDER, Beitr. z. Klin. d. Tuberk. **79**:98, 1931.

For measuring the amount of lipase in sputum the titrimetric method is best. One hundred sputums obtained in cases of tuberculosis were examined, and it was found that in all cases the amount of lipase was minimal, but that it increased somewhat in severe cases. Since in severe cases the lipase content is decreased in serum it is assumed that the decrease is due to excretion of the lipase through the sputum.

MAX PINNER.

GROUP-SPECIFIC REACTIONS OF THE STROMA OF RED CORPUSCLES. F. OTTENSOOSER and ST. ZURUKZOGLU, *Klin. Wchnschr.* **17**:719, 1932.

The group reactions of the stroma, according to Dold and Rosenberg, corresponded in 85 per cent of instances with the results of the usual tests for blood groups. An improved modification of the method, as well as a new method, is described. As a rule, the stroma gives a lower titer of agglutination than the corpuscles, but on preservation the stroma remains, in most instances, more agglutinable than the corpuscles.

FROM THE AUTHORS' SUMMARY.

IMMUNITY IN RABIES. E. LOEFFLER and F. SCHWEINBURG, *Virchows Arch. f. path. Anat.* **279**:181, 1930.

The results of the experiments described failed to support the older theory of the formation of antibody within the brain in rabies. Immunity could not be produced by intracerebral injection of fixed virus, but followed intramuscular, intraperitoneal and subcutaneous injection. The findings indicate that the formation of antibody occurs, not within the brain, but in the other tissues of the body.

W. SAPHIR.

ACTIVE IMMUNIZATION AGAINST DIPHTHERIA. M. P. ISABOLINSKI, B. P. KARPATSCHEWSKAJA and W. F. TOWJANSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:27, 1931.

Active immunization with Ramon's anatoxin lowered the morbidity from seven to ten times in 4,185 children. The local and general reactions were of no significance. The percutaneous method of Loewenstein is strongly recommended.

I. DAVIDSOHN.

PRODUCTION OF ANTIBODIES AGAINST ORGANS OF THE HOMOLOGOUS SPECIES. S. SCHILLING-SIENGALEWICZ and W. BIELOSZABSKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:31, 1931.

The cold precipitation technic of Kumagai and Yanabashi was checked and found correct. In addition to the already established serologic relationship between the kidney and the liver, such a relationship was found between the latter organ and the thyroid gland.

I. DAVIDSOHN.

THE BLOOD ANTIGENS IN *BACILLUS PARATYPHOSUS B* AND *BACILLUS DYSENTERIAE SHIGA*. M. EISLER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:37, 1931.

*B. paratyphosus B* and *B. dysenteriae Shiga* contain heterophilic (Forssman) antigen. Only the homologous bacteria are able to absorb the antishoop hemolysin produced by them, but they are unable to bind the hemolysins produced by sheep red cells and by guinea-pig tissues. The heterophilic antigen in *Shiga bacilli* is contained in their carbohydrate fraction; that of paratyphoid *B bacilli*, in their watery and alcoholic extracts, but not in their carbohydrate fraction. The carbohydrate fraction of *Shiga bacilli* contains also a human blood antigen (M-antigen) showing no group specificity; paratyphoid *B bacilli* contain an alcohol-soluble antigenic substance related to the human A-receptor.

I. DAVIDSOHN.

THE TWO TYPES OF THE BLOOD GROUP QUALITY A IN MAN. M. AKUNE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:75, 1931.

The A-large and the A-small type of the blood group A can be differentiated with the help of hetero-antibodies as well as with iso-antibodies. The differences are preserved in aqueous and alcoholic extracts of the red blood cells and are

present in the blood serum, saliva and urine. Red blood cells of type A-large more frequently produce group-specific antisera in the rabbit than those of type A-small; specific antisera for the A-small red blood cells could not be obtained.

I. DAVIDSOHN.

SUBSTANCES STIMULATING PERISTALSIS AND LOWERING BLOOD PRESSURE IN ORGANS OF SENSITIZED AND SHOCKED DOGS. K. WATANABE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:149, 1931.

An increase of the substances mentioned in the title was observed in livers of sensitized animals followed by a marked drop during anaphylactic shock. No such changes were found in the other organs, except the spleen, which showed a marked increase both in sensitized and in shocked dogs.

I. DAVIDSOHN.

AGGLUTINATION OF SHEEP RED BLOOD CELLS BY HUMAN SERUM IN COMPLEMENT-FIXATION TESTS. OSCAR FISCHER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:154, 1931.

A marked agglutination of sheep red blood cells was observed in a positive complement-fixation test for gonorrhea. Agglutination was not present and hemolysis took place when syphilitic antigen was employed. In the latter case free complement was available. Agglutination occurs only when the complement is fixed. Agglutinins for sheep red cells do not interfere with hemolysis in the presence of complement.

I. DAVIDSOHN.

THE CONCENTRATION OF ANATOXIN. W. M. KULIKOW and A. W. BEILINSON, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:178, 1931.

The precipitation of proteins and of related nitrogenous substances was accomplished with hydrochloric acid in the presence of certain anticoagulants. Four fifths of the nitrogenous substances and all the solids were removed in this manner. The anatoxin sediment was easily soluble and produced no local reactions in the guinea-pig.

I. DAVIDSOHN.

THE RÔLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNITY. B. P. KARPATSCHEWSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:190, 1931.

Trypan red, india ink and phenylhydrazine were used for the blocking of the reticulo-endothelial cells before and after immunization of guinea-pigs with diphtheria anatoxin. The antibody response was adversely affected, particularly when india ink was used.

I. DAVIDSOHN.

IMMUNOLOGIC STUDIES IN PNEUMOCOCCIC INFECTIONS. M. GUNDEL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:193, 1932.

Serums of 126 persons of various ages were examined for type-specific anti-pneumococcal protecting substances and agglutinins. They included healthy persons and such as were suffering from various pneumococcal infections. In 5 of 15 normal adults with no history of previous pneumococcal infection, considerable quantities of such substances were found. They were absent in 12 healthy newborn infants and young children. In patients suffering from bronchopneumonia and from pneumococcal infections of the upper respiratory tract, type-specific protecting substances and agglutinins could not be detected. They were found in large quantities in persons with lobar pneumonia and postpneumonic empyema due to type I and type II pneumococci. They appeared infrequently before the crisis, but reached their maximum during convalescence. In type III infections,

antibodies were rarely produced. Children till the end of the second year are poor producers of antibodies. In otitis media and in peritonitis due to pneumococci, the development of protecting substances and less frequently of agglutinins was observed, while the serums of type I and type II pneumococcus carriers were devoid of protecting and agglutinating qualities. The use of convalescent serums for therapeutic purposes is advocated. Slight development of antibodies was observed in children about 1 year old and older, following vaccination with killed pneumococci.

I. DAVIDSOHN.

THE MECHANISM OF COMPLEMENT FIXATION AND OF THE PRECIPITATION REACTION. D. P. BOROWSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:239, 1932.

In the Meinicke reaction and a slightly modified citochol reaction, the complement of the active serum is not affected by the flocculation, which takes place in a hypertonic medium. The natural antisheep hemolysin was also found in the supernatant fluid after the precipitate was removed by centrifugation. It is concluded that flocculation and complement fixation do not always run parallel, at least not in a hypertonic medium.

I. DAVIDSOHN.

SEROLOGIC DEMONSTRATION OF BRAIN ANTIGEN IN THE BLOOD. F. PLAUT and H. RUDY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:242, 1932.

Serum of rabbits fed with brain substance or given subcutaneous injections of it did not react in the complement-fixation test with brain antiserum. Reaction occurred when large quantities of the antigen were injected intravenously. The lack of reaction is due to the masking effect of serum lipoids on the injected antigen, as shown in vitro. The acetone-insoluble fraction of the serum lipoids seems to be the factor responsible for the masking. The antigenic effect of the brain lipoids reappeared following removal of the acetone-insoluble fraction.

I. DAVIDSOHN.

GLUCOSIDES AND VACCINATION WITH VIRULENT BACTERIA. K. HRUŠKA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:256, 1932.

Virulent anthrax bacilli and spores suspended in tissue irritants, saponin and digitonin, can be injected subcutaneously into rabbits and large animals with resulting immunity. No such protection is obtained in guinea-pigs. The local necrosis produced by the irritants prevents the generalization of the infection. The virulence of the bacilli kept for a long time in the solutions of saponin and digitonin is not affected. Proper concentration and large doses of the irritants are essential, as in case of too small quantities the insufficient local reaction is unable to stop the spread of the infection. The glucoside of the chestnut (*Rosskastanie*) has a local necrotizing effect, but lacks the protecting property.

I. DAVIDSOHN.

COMPLEMENT FIXATION WITH SOLUTIONS OF RED BLOOD CELLS. E. BRUNIUS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:293, 1932.

The solutions were employed for the demonstration of the Forssman antigen in the red cells of the guinea-pig with equally good results as alcoholic extracts. Heating to 100 C. destroyed their complement-fixing ability, which reappeared in alcoholic extracts of the same heated solutions. Species and group-specific antigens were retained in such solutions of red cells of man and pig. The group-specific qualities could be better demonstrated with the help of the complement-fixation test, in which the dissolved red blood cells were used as an antigen, than when the agglutination with native red blood cells was employed. However, alcoholic extracts are still better for demonstration of group specificity.

I. DAVIDSOHN.

INACTIVATION OF AGGLUTININS BY DIGESTIVE SECRETIONS. A. A. SCHMIDT and KLARA TULJTSCHINSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:312, 1932.

Agglutinins for *Bacillus typhosus* were destroyed by active, and to a lesser extent by heated gastric juice. The hydrochloric acid is probably an important factor, as it effects the same result by itself. Active pancreatic secretion had a weaker effect, while active intestinal ferments did not harm agglutinins.

I. DAVIDSOHN.

## Tumors

THE METABOLISM OF TUMOR CELLS. G. SCHMIDT, Frankfurt. *Ztschr. f. Path.* **41**:393, 1931.

This article deals with the catabolic and anabolic properties of tumor cells, especially with the process of glycolysis. The more important literature on the subject is reviewed, but original observations are not given. The author points out that it still remains a matter of further research to study quantitatively synthetic processes of tumor cells.

O. SAPHIR.

CARCINOMA OF THE PROSTATE GLAND AND CRYPTORCHISM IN A DOG. C. KRAUSE, Frankfurt. *Ztschr. f. Path.* **41**:405, 1931.

In an 8 year old dog in which the left testis had been removed six years previously, the right testis and epididymis were found in the right iliac region. The surface of this testis appeared irregular, granular and hemorrhagic. A few dark red areas of softening were found in the center. The prostate gland was almost completely transformed into two vesicle-like structures. Parts of the walls of these vesicles were necrotic, crossed by firmer bridges of tissue recognized grossly as tumor. The urethra contained many undermined ulcers in addition to circumscribed thickenings. In the histologic sections of the testis, accumulations of cells were arranged in the form of alveolus-like structures, some of which were completely filled with tumor cells. Some of these cells were large, with vesicular nuclei and much cytoplasm; others small, resembling lymphocytes; a few polygonal, containing round nuclei. There were only few mitotic figures. The prostate gland microscopically was composed of tubular structures, masses of epithelial cells and cystlike structures, in addition to many inflammatory cells.

The author, who states that the finer histologic details will be given in another paper, concludes that the tumor of the testis is neither characteristic of carcinoma or sarcoma, but should be classified as a precancerous condition. The tumor of the prostate gland is classified as carcinoma.

O. SAPHIR.

GROUP-SPECIFIC AND HETEROGENEOUS STRUCTURES IN THE LIPOID ANTIGEN OF MALIGNANT TUMORS. H. LEHMANN-FACIUS, Frankfurt. *Ztschr. f. Path.* **41**:521, 1931.

This detailed article deals mainly with problems of method in the study of specificity of immune serum in cases of malignant tumors.

O. SAPHIR.

PLEXIFORM NEUROMA WITH PACINIAN CORPUSCLES. M. BRÖGLI, Frankfurt. *Ztschr. f. Path.* **41**:595, 1931.

Such a tumor is described in the lumbar region of a 16 year old youth. The author believes that this is the first case reported of a neuroma in which typical pacinian corpuscles were found.

O. SAPHIR.

EFFECT OF ROENTGEN RAYS ON THE METABOLISM OF JENSLN SARCOMA. A. LÓW-BEER and M. REISS, *Strahlentherapie* 42:157, 1931.

The metabolism of slices of irradiated Jensen sarcoma was examined with the Warburg method. It was found that there was a shift from the oxidative to the fermentative type of metabolism. In normal tissues this shift occurs later and after higher doses of x-rays. This difference in response is attributed to differences in radiosensitivity.

WILHELM C. HUEPER.

VARIATIONS IN THE LACTIC ACID CONTENT OF THE BLOOD AFTER ROENTGEN TREATMENT. J. HEEREN and R. HUMMEL, *Strahlentherapie* 42:784, 1931.

Roentgen irradiation of the tumor resulted in an increase in the lactic acid of blood removed from six to eight minutes after treatment (an increase of from 22.9 to 102 per cent) in sixteen of nineteen cases. After irradiation of the thigh muscles of the same patients (six), a definite decrease in the lactic acid (of from 7.8 to 44 per cent) was seen. Similar results were obtained in noncarcinomatous persons (decrease in lactic acid of from 10 to 15.6 per cent). The location of the tumor had no effect on the result obtained. Irradiation of the hypochondric and splenic region did not change these results. The increase in lactic acid of the blood in patients with cancer after irradiation of the tumor is therefore not an indirect effect.

WILHELM C. HUEPER.

THE PATHOGENESIS OF CARCINOIDS. F. FEYRTER, *Verhandl. d. deutsch. path. Gesellsch.* 26:286, 1931.

The carcinoids of the human intestinal tract are not congenital malformations, but true new growths. Fifty-one of seventy-five carcinoids studied by the author were located in the jejunum and ileum. In this portion of the intestines, the carcinoid cells are related to the so-called yellow cells of Schmidt, while those in the duodenum and stomach must have another origin, since they are not argentaffin and not chromaffin. They resemble more the cells in Langerhans' islands and the peculiar clear cells that are normally found in the surroundings of the excretory duct of the pancreas.

C. ALEXANDER HELLWIG.

THE PATHOGENESIS OF ADIPOSITY IN CARCINOMA. E. MATHIAS, *Verhandl. d. deutsch. pathol. Gesellsch.* 26:289, 1931.

Some patients with carcinoma show, in spite of long duration of the disease, a high grade of adiposity. Wohlwill found that such patients may have metastases of the cancer in the pituitary gland or its surroundings. Considering only patients in whom the subcutaneous tissue of the anterior abdominal wall had a thickness of from 6 to 8 cm., Mathias found metastatic carcinoma in the hypophysis in a few. In the majority of his cases, however, this explanation did not hold. The author believes that the continuous loss of blood in carcinomas of the stomach, rectum and cervix or in multiple metastases in the bone marrow is responsible for severe anemia, and that the resulting anoxemia is the causative factor of adiposity in these cases.

C. ALEXANDER HELLWIG.

INFLUENCE OF LOCAL CIRCULATORY DISTURBANCE AND ANEMIA ON THE GROWTH OF TRANSPLANTED TUMORS. B. MORPURGO, *Verhandl. d. deutsch. path. Gesellsch.* 26:292, 1931.

An osteoid sarcoma was implanted into one kidney of each of a number of white rats. In one group of these animals, active hyperemia of the kidney containing the engrafted tumor was produced by removing the other kidney. In the second group, the blood vessels of the kidney containing the tumor were ligated, to produce permanent anemia. In both experimental series the graft survived, but

in the hyperemic kidney the transplanted tumor reached a much larger size than in controls, and in the anemic kidney the rate of growth was much slower. To study the influence of general anemia, osteoid sarcoma was implanted under the skin of white rats and severe anemia produced by frequent bleeding of the animals. In the anemic rats the rate of growth of the transplanted tumor was not less active than in normal animals, and also the duration of life was not shortened by the general anemia.

C. ALEXANDER HELLWIG.

LIPOID CONTENT OF MALIGNANT TUMORS. R. BIERICH, A. DETZEL and A. LANG, *Ztschr. f. physiol. Chem.* **200**:157, 1931.

Malignant tumors have relatively high phospholipoid and cholesterol ester. In malignant tumors, the quotient phospholipoid:cholesterol ester is small, while in normal tissues it is high. Normal tissues contain relatively small amounts of both substances. There was no increase or decrease in the lipoid of the tissues surrounding the tumor. Benign tumors have lower cholesterol ester and phospholipoid than malignant ones. The quotient is, however, the same as in malignant tumors.

WILHELM C. HUEPER.

### Medicolegal Pathology

CHANGES OF THE HYPOPHYSIS AND THE CHIASM IN SEVERE TRAUMA. C. F. PORTA, *Beitr. z. gerichtl. Med.* **11**:83, 1931.

This study is based on observations of thirty-five cases. In thirty-two, the skull was fractured, nine of these revealing complete destruction of the base of the skull. In three instances, there were only contusions of the brain. The author concludes that rupture of the hypophysis occurs only in cases in which there is complete destruction of the skull. Such a condition is incompatible with life. Gross and microscopic changes of the hypophysis due to trauma are, in general, rare. Hemorrhages and contusions, which sometimes occur, are located in the center of the anterior lobe. Changes in the chiasm resulting from trauma are only occasionally encountered. A relation between trauma and clinical symptoms referable to lesions of the hypophysis should be regarded only with the greatest reserve. Such a relation might exist in cases of very severe trauma.

O. SAPHIR.

STRUCTURAL CHANGES IN CHRONIC POISONING WITH ARSINE. K. BEÖTHY, *Beitr. z. gerichtl. Med.* **11**:97, 1931.

Two dogs were fed with from 6.305 to 6.398 mg. of arsine per kilogram of body weight over a period of three months. Most commonly, recent and old hemorrhages were found in the thymus, stomach, small and large intestines, spleen, kidneys, lungs, lymph nodes and mucosa of the urinary bladder. None were found in the subendocardial spaces. Peyer's patches and solitary follicles, which contained only mature lymphocytes, were edematous and enlarged. Macrophages were found in the spleen. The intestinal tract revealed the most severe inflammatory changes.

O. SAPHIR.

LARYNGEAL WOUNDS IN THE NEW-BORN INFANT DUE TO ATTEMPTS AT REVIVAL. A. FELLER, *Beitr. z. gerichtl. Med.* **11**:150, 1931.

In two instances, endolaryngeal wounds in asphyxiated new-born infants were produced by the introduction of tracheal catheters for the purposes of revival. In each instance the trauma was in the anterior wall of the larynx corresponding to the ligamentum conicum. The autopsy also disclosed subcutaneous emphysema, which extended into the mediastinum. One of the infants revealed, in addition, bilateral pneumothorax.

O. SAPHIR.

TWO RUPTURES OF THE LEFT VENTRICLE FROM INTERNAL CAUSES. L. BREITENECKER, *Beitr. z. gerichtl. Med.* **11**:181, 1931.

In one instance, two areas of myomalacia were found in the anterior wall of the left ventricle, both of which had ruptured, with resulting hemopericardium. There was marked arteriosclerosis involving the main branches of the left coronary artery. In the second instance, two points of rupture were demonstrated in one large area of the myocardium situated in the posterior wall of the left ventricle, likewise with hemopericardium. The descending branch of the right coronary artery was occluded by calcified plaques.

O. SAPHIR.

SPONTANEOUS DIFFUSE SUBARACHNOIDAL HEMORRHAGE. O. HESS, *Deutsche med. Wchnschr.* **58**:45, 1932.

It is claimed that diffuse subarachnoidal hemorrhage may occur spontaneously in healthy persons, especially young persons, with a normal vascular system. Such hemorrhage may be due to vasoneurotic disturbances of the leptomeningeal vessels, but in every case most careful search should be made for structural vascular changes and particularly for basal aneurysm.

FINDINGS OF DIAGNOSTIC VALUE ON BURNT AND CHARRED HUMAN BODIES. HERMANN MERKEL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:232, 1931.

Destruction of a human body by fire offers great difficulties in correct interpretation. Roentgenologic examinations of the collected parts of the débris may disclose presence of bone fragments. For the differentiation between human and animal bones, the biologic tests, either on the still attached tiny particles of flesh or with ground bone, may be successfully employed. The teeth may be of valuable assistance in identification. While amalgam and gold dental work may be destroyed by fire, porcelain material resists the highest temperatures, as much as from 700 to 900 C., and has been found even in the ashes of cremated bodies. The most important question to decide is: Was the person burnt while alive or dead? Evidence of carbon monoxide in the internal organs, as in the blood of the cardiac cavities and in the aorta, liver, abdominal veins, etc., is conclusive evidence of existence of life during the fire, while presence of carbon monoxide in the blood vessels or tissues beneath the surface of the body alone may occur after death if the body was exposed to this gas in an enclosed environment. Absence of carbon monoxide in the organs does not exclude the possibility that the person was alive at the time of the exposure to fire, since psychic shock may lead to a sudden collapse, or aspiration of vomitus in the initial stages of carbon monoxide poisoning may cause death from asphyxiation. Presence of soot or smudging in the bronchial tree may serve as an additional diagnostic sign. An epidural hemorrhage or hematoma may develop in dead bodies under the influence of intense fire, and, if the head was fractured or crushed by falling débris after death, one may be deceived into assuming a vital injury. Stab wounds usually appear considerably smaller, owing to shrinkage of the drying skin. Destructures of the extremities are not uncommonly observed and then charred stumps of long bones may project freely. Extensive fracturing of bones that possess a well developed marrow space occurs on account of an explosive action. Fracture of the thyroid cartilage may be an artefact of the fire and should be carefully evaluated as a sign of strangulation. Microscopic evidence of embolism of liver cells or brain tissue is undoubtedly a positive proof of intravital injuries. Presence of minute fat globules in the territory of the pulmonary artery was found by several observers and is interpreted as evidence of vital injuries prior to the destruction of the body by fire.

E. L. MILOSLAVICH.



IDENTIFICATION OF BURNT BODIES BY MEANS OF THE TEETH. K. BOEHMER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:250, 1931.

Identification of a human body destroyed by fire is a most difficult task. However, it is often achieved by an accurate examination of the teeth with the characteristic findings thereon, since they resist extreme temperatures and processes of decomposition. A pertinent case is presented in minute detail, and the mode of procedure is illustrated. A caoutchouc (india rubber) plate containing an upper incisor was not destroyed by the fire, contrary to the opinion of Gebhardt, who assumed that this material succumbs readily. Prolonged exposure to high degrees of temperature may cause calcination of the teeth, particularly of the exposed incisors, but they do not fall apart; their structure remains still evident. Pieces of enamel may occasionally crack and break off, the edges of the broken-off area appearing angular and sharp in contrast to a carious area, which is more or less round and smooth-edged. All the teeth in every decade of life present the same behavior toward fire; the front teeth, however, being exposed and anatomically unprotected, are most readily attacked. Teeth on which dental work has been done are more resistant than others. Experiments on teeth by exposing them to fire disclosed some interesting data. Fillings of silicate cement and crown cement became more hard, and consequently these teeth appeared less cracked than the unrepaired ones. Silver amalgam withstood only lower temperatures, while gold-platinum amalgam resisted higher degrees of and longer exposures to fire. Copper amalgam showed the strongest resistance.

E. L. MILOSLAVICH.

DEATH BY EXPOSURE TO COLD. KARL MEIXNER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:270, 1931.

Clinical observations made on a 68 year old man who was found in the Tyrolian Alps, frozen but still alive, are interestingly presented. Respiration was noticeable, but the body was pulseless and apparently without heart beat. The lower part of the legs and both feet were hard and completely frozen. After energetic attempts at revival, the man, as in a daze, mumbled a few words and died fifteen hours later. Autopsy showed a slight lysis of the blood, but otherwise the erythrocytes were intact. Microscopic examination of the frozen legs disclosed the most marked changes in the epidermis and subcutaneous blood vessels. The external layers of the epidermis down to the stratum lucidum appeared as if exfoliated. The germinative layer presented greatly changed nuclei, which were either shrunk and then surrounded by a light, ringlike space, or swollen and in stage of lysis. The blood vessels were contracted or collapsed, and their endothelial elements were present sparsely and without nuclei. Fibrin was not evident. The striated muscles presented a pronounced crumbling of their protoplasm in places, while in the adjoining portions the striation was present. The cells of the intramuscular fat tissue contained needle-like, yellowish-brown crystals. They apparently developed on account of action of dissolved coloring matter of the blood and muscle tissue on the fat substance. The success of resuscitation depends on the length of time of exposure to cold, since the greatly lowered circulation of the blood produces irreparable damages in the vital centers. Meixner cites the case of a 25 year old nurse who, in a suicidal attempt, ingested morphine and veronal and was found, cold and apparently dead, in a park. A physician pronounced her dead, but the next morning, after she had lain for fifteen hours in a coffin at the morgue, it was discovered that she was alive. Resuscitation was successful, and her life was saved. These and similar cases are an earnest warning against attesting a body dead, in instances of exposure to cold, without a conscientious and thorough examination, as one may be confronted with only an apparent death.

E. L. MILOSLAVICH.

TEARING OF THE MUCOUS MEMBRANE OF THE STOMACH IN DEATH BY DROWNING. ERICH FRITZ, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:285, 1931.

In four cases of death due to drowning, linear lacerations of the gastric mucous surface along the lesser curvature and cardia were found, but there was no evidence of external violence. In one instance, the tears were located mainly around the cardiac opening, and in two the proximal third of the lesser curvature was involved. In the fourth case, which showed a tearing also of the serosal covering, the distal third was affected. In all the cases, punctate hemorrhages were present, not only within the torn tissues, but also in the immediate vicinity of these tissues, indicating that the tearing happened during life. A traumatic rupture of the stomach may be the result of direct external violence, and then it occurs chiefly close to the pylorus or on the anterior wall of the stomach. In none of the thirteen cases described by Rehn was the lesser curvature found involved. The mechanism of the tearing during drowning is explained by the increase of intragastric pressure. Vomiting, which is frequently observed in drowning, produces a contraction of the pyloric portion of the stomach, while the cardiac area undergoes a partial distention, thus leading to a tearing of the mucous surface around the cardiac mouth.

E. L. MILOSLAVICH.

AN UNUSUAL, NOT PREVIOUSLY REPORTED CADAVERIC CHANGE. PHILLIP SCHNEIDER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:325, 1931.

Changes of the eyeballs occur soon after death and consist of an opaque appearance and drying up of the external surface, and loss of tension, causing collapse of the bulbi. If the vitreous body liquefies, the eye may appear as a flabby sac. In a woman, aged 48, who died of carcinoma of the uterus with peritoneal metastases, both eyeballs showed, a few hours after death, complete liquefaction and had disappeared altogether. The orbital cavities were filled with a dark-colored, bloody fluid containing gas bubbles, and this fluid material was running down the cheeks. Culturally, *Bacillus mesentericus*, *Bacterium coli* and hemolytic streptococci were obtained from the orbital contents. This peculiar cadaveric phenomenon is explained as the effect of terminal bacteremia causing putrefactive destruction of both eyes.

E. L. MILOSLAVICH.

OCCUPATIONAL PNEUMOCONIOSIS IN AN EXHUMED BODY. E. ZIEMKE, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:330, 1931.

Autopsy on an unembalmed body ten weeks after interment clearly demonstrated pneumoconiotic changes. The roentgenographic studies of the lungs during life, made by experienced specialists, were not conclusive as to presence or absence of coniotic lesions, hence the value of postmortem examination in such instances is emphasized. The microscopic changes are presented in detail, and the question of a macronodular type of silicosis in its relation to pulmonary tuberculosis is mentioned.

E. L. MILOSLAVICH.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, May 26, 1932*

CHARLES T. OLCOTT, *Vice-President, in the Chair*

### ECHINOCOCCUS CYST FORMING THE CONTENT OF VENTRAL HERNIA. LAWRENCE H. COTTER.

A laborer, 30 years old, born near Naples, had always worked in a vineyard until coming to this country about ten years ago. About eight years ago he noticed a ventral hernia, which soon reached its maximum size. This hernia caused him little trouble and was easily reducible. He kept it in place by wearing high-waisted trousers and at times also wore a bandage around the abdomen. On the day of his admission to the hospital, the mass became very painful, and he was unable to reduce it as he had previously.

Physical examination showed a man suffering from acute pain. There was a tumor mass in the midline of the abdomen, above the umbilicus, which was tender on pressure and not reducible. It was about 10 cm. in diameter.

At operation, a cystic mass about 10 cm. in diameter was found to be the content of the hernia. The cyst was attached to the left lobe of the liver. It was filled with clear fluid, and contained five daughter cysts, each about 2 cm. in diameter. The daughter cysts were free in the fluid of the parent cyst. The fluid contained numerous hooklets and scolices.

### PATHOLOGY OF HEMOLYTIC JAUNDICE. WILLIAM P. THOMPSON (by invitation).

A study of the clinical and pathologic findings in thirty cases of hemolytic jaundice is reported. Of these thirty cases, twenty-six presented the usual clinical findings associated with the congenital type of this disease, although in many of the twenty-six cases evidence of the congenital or familial nature was lacking.

The spleens in these twenty-six cases presented a uniform and characteristic appearance. They weighed from 1,000 to 1,500 Gm., and presented a homogeneous purple appearance on section. The microscopic appearance was uniform in all instances. The malpighian bodies were small and widely separated. The venous sinuses were enlarged, widely dilated and frequently empty. The endothelium of the sinuses was prominent, the outline of the cells often bulging into the lumen. The pulp was a mass of closely packed red cells, and this diffuse infiltration was the most striking microscopic feature. There was neither increase in connective tissue nor visible increase in iron pigment. The so-called iron incrustations were frequently encountered, lying usually within the trabeculae. There was no visible evidence of phagocytosis of the red cells, and except for an occasional lymphocyte, one found nothing but red cells distending, distorting and dilating the pulp.

It is of interest in this connection that in a review of a large number of splenomegalies of various sorts, the only ones presenting this picture were in cases of this disease. It is also of importance that, of two spleens presenting this appearance, one was from a patient with the diagnosis of pernicious anemia, and the other from one with the diagnosis of Banti's disease. Subsequent clinical investigation revealed the fact that both patients had hemolytic jaundice of

the congenital type. In both instances, the diagnosis was made first by finding the characteristic features of the disease in the microscopic section of the spleen. In both instances, this diagnosis was confirmed by subsequent clinical investigation.

## DISCUSSION

MAURICE N. RICHTER: Was any distinction made between the congenital and the acquired type of the disease?

WILLIAM P. THOMPSON: We have included under the so-called congenital type all the typical cases of hemolytic jaundice. Some of them apparently went back to childhood; some were associated with a familial incidence of the disease, and others were not. As far as we could see, the disease was typical in all the cases, whether congenital or acquired.

MAURICE N. RICHTER: Did you have any very young patients?

WILLIAM P. THOMPSON: The youngest was 9 years old.

## UNUSUAL METASTASES OF MALIGNANT TUMORS. REPORT OF SIX CASES. ANGELO M. SALA and (by invitation) ELMA BARANY.

Two cases of carcinoma of the cervix are presented, with metastases in the lungs. One was in a 60 year old woman with a highly malignant anaplastic carcinoma; the other, in a 28 year old woman with a transitional cell carcinoma. Both patients were colored. The metastases in the lungs were found at autopsy.

The third case was that of a white man, 55 years old, with an epithelioma of the pharynx. Routine examination of sections of the left kidney post mortem disclosed an unsuspected metastasis from the pharyngeal growth.

The fourth case, also an epithelioma of the pharynx, was in a man 64 years old. Postmortem examination disclosed metastases to the liver and lungs.

The fifth was a case of recurrent epithelioma of the penis in a white man 36 years old. At autopsy there was found diffuse thickening of the intestinal serosa and of the splenic capsule. Grossly there was no suggestion of malignancy, but microscopically the picture was that of metastatic deposition (fig. 1).

The sixth case was an epithelioma of the tongue, with bilateral metastases to the cervical nodes, in a colored man 49 years old. At autopsy, extensive involvement of heart muscle was found. Two of the metastatic tumors were 3 cm. and 2 cm. in diameter, respectively. Smaller deposits were scattered throughout the myocardium, apparently following a coronary distribution (fig. 2).

These six cases, showing some of the decidedly less usual metastases, illustrate the necessity of painstaking search for evidences of metastasis in all cases of malignant growth. The first two cases offer an interesting contrast. In a 60 year old woman one does not usually see a rapidly growing anaplastic carcinoma of the cervix with distant metastases. In the second case, the cervical cancer was of the type usually encountered, from which one does not ordinarily expect distant metastases, except very late after onset. That both patients were colored may be of some significance. In the third case, renal metastasis from an epithelioma of the pharynx was neither expected nor suspected, and the discovery of it on our part must be regarded as a fortunate accident. Metastasis to the pulmonary parenchyma from an epithelioma of the pharynx, as presented in the fourth case, is certainly not common. The fifth case is remarkable because of the unusual dissemination of the metastases, manifesting themselves as a diffuse, not nodular, thickening of the intestinal serosa and splenic capsule. Only the microscope disclosed the true state of affairs in this case. Finally, metastasis to the heart from an epithelioma of the tongue is an extremely rare occurrence, and the case here reported is a brilliant example of the capricious behavior of malignant tumors with which we are at times confronted.

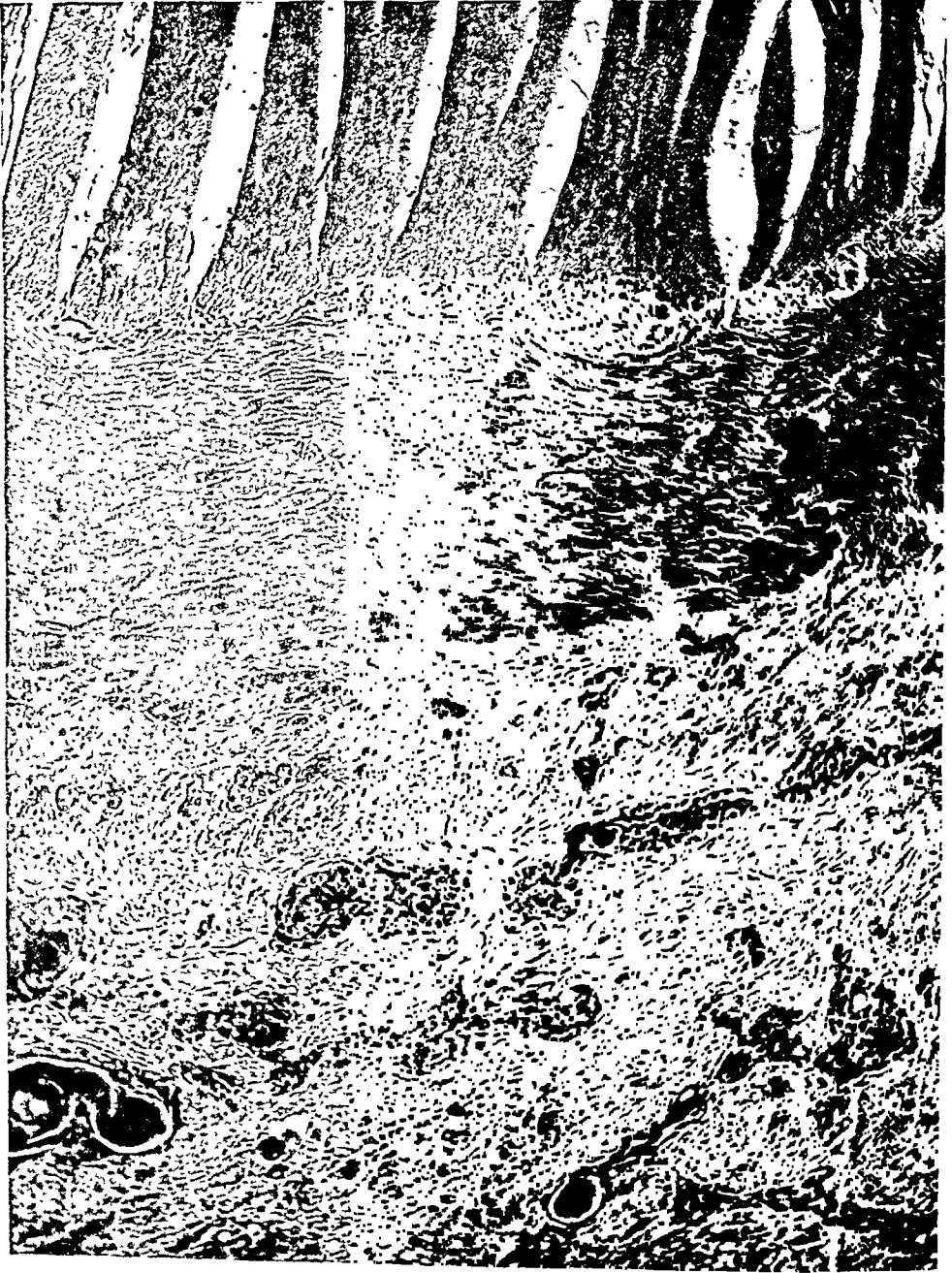


Fig. 1.—Intestinal serosa with metastatic squamous cell epithelioma; reduced from  $\times 140$ .

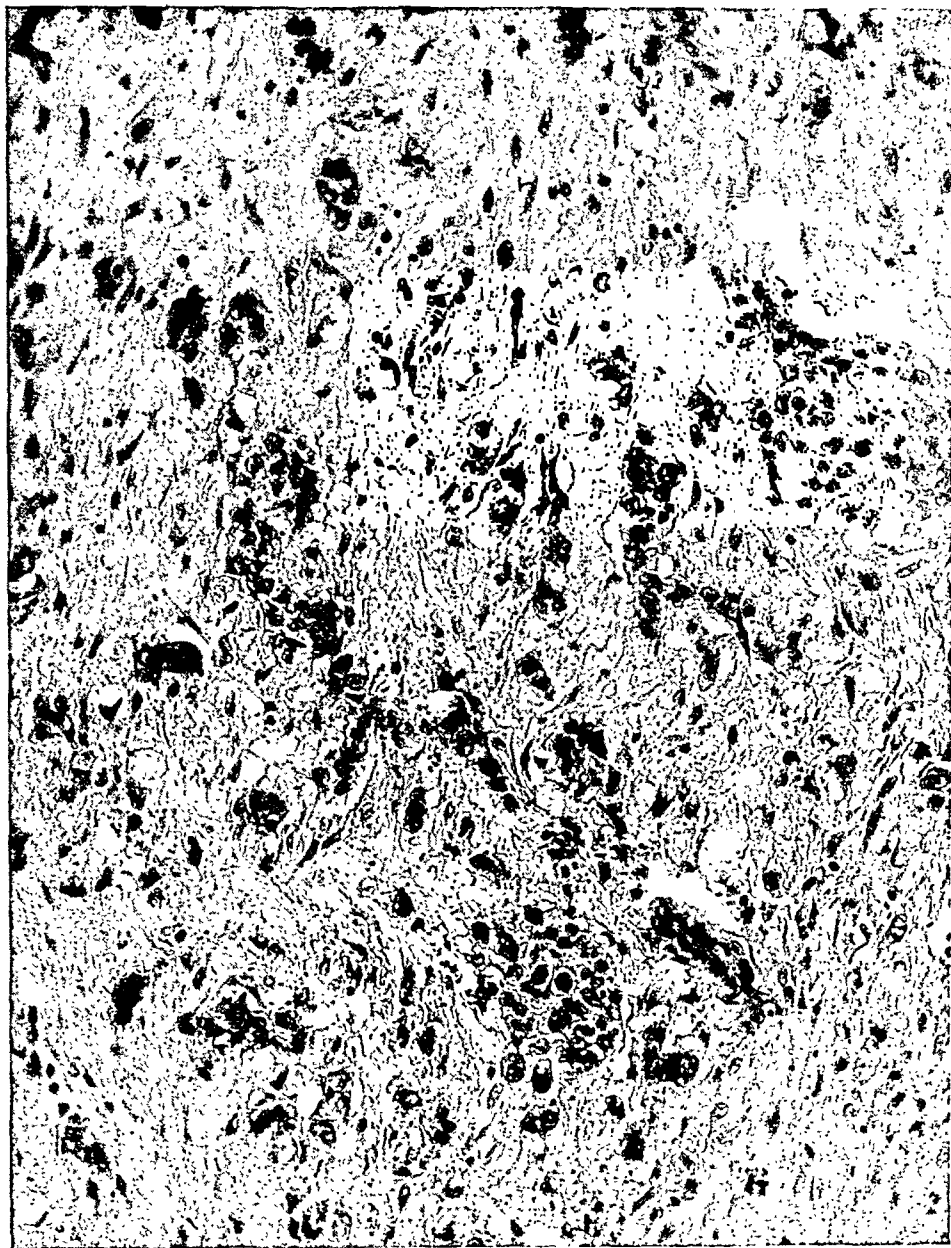


Fig. 2.—Metastatic squamous cell epithelioma in myocardium; reduced from  
X 280.

## FACTORS INFLUENCING TYPES OF BONE RESORPTION. HENRY L. JAFFE and AARON BODANSKY.

Four litters of dogs, 3, 6, 8 and 18 months old, respectively, were given gradually increasing doses of ammonium chloride. Some of the litter mates in each age group received a diet in which calcium was high; others, a diet in which calcium was low. Some of those on each diet were given ammonium chloride, and some were retained as high and low calcium controls without ammonium chloride. The diet consisted of fresh lean horse meat supplemented in the case of the dogs receiving the diets in which calcium was to be high, with bone meal and calcium lactate (2.5 Gm. of each per kilogram of body weight), and in all cases with cod liver oil and tomato juice. Thus the calcium supplement amounted to from 0.5 to 2.5 Gm., depending on the size of the dog and on its food consumption. Once a week all dogs were fed cooked liver and kidney. The ammonium chloride, in 1 per cent solution, was given by stomach tube once a day at the beginning of the experiment; later, in order to avoid giving excessive volume at one time, the ammonium chloride was given twice a day. At the end of the experiment as much as 1 Gm. per kilogram was being given daily.

The animals were subjected to the experiment for eleven weeks. In the control dogs receiving the calcium supplement, the bones were normal in every respect. The low calcium controls showed thinning of the bones. The effects of the ammonium chloride treatment were observed in all the age groups, but were most striking in the younger animals. In a dog subjected to the experiment from the age of 3 months, a low calcium diet plus ammonium chloride led to fractures and deformities. Less striking changes were observed in a litter mate given ammonium chloride plus a high calcium diet.

In all age groups the gradation of changes was found to be strikingly dependent on the calcium intake. Those dogs receiving a diet in which calcium was high and ammonium chloride showed less osteoporosis than those receiving a diet in which calcium was low plus ammonium chloride or those receiving this diet without ammonium chloride. In the younger age groups a diet low in calcium to which ammonium chloride was added brought out more striking changes than a low calcium diet alone.

## A CASE OF ACUTE MYELOID LEUKEMIA WITH UNUSUAL FEATURES. EDITH E. SPROUL (by invitation).

A 39 year old Englishwoman was admitted to the Presbyterian Hospital in a febrile condition, with a severe streptococcic lesion of the throat of one week's duration. The white count, at that time, totalled 2,500 cells per cubic centimeter, with the following differential count: polymorphonuclear leukocytes, 7 per cent; lymphocytes, 44 per cent, and unclassified cells, 47 per cent. The latter cells closely resembled the monocyte in nuclear configuration and inner pattern, but bore the cytoplasm of the myeloblast. Supravital staining revealed none of the rosettes of the monocyte. Peroxidase stains were positive.

The known course of the disease occupied twenty-seven days. There was a terminal rise in the white count to 89,000, with a differential count of myeloblasts of 47 per cent, and of myelocytes, 44 per cent; no eosinophils were noted. The spleen became enlarged and painful. Death followed rapid collapse, with an abrupt fall in the red cell count.

Postmortem examination established the immediate cause of death as hemorrhage into the peritoneal cavity following rupture of a large subcapsular hematoma of the spleen. There was bilateral lobular pneumonia with small abscess formation. Grayish foci of infiltration were visible grossly in the liver and kidneys. The lymph nodes were but slightly enlarged and soft. The thyroid gland was large. The bone marrow everywhere appeared dark red and moist.

Microscopically, the distribution of the visceral infiltration was not remarkable except for a predilection for the perivascular zones and an unusual intensity in

the portal areas of the liver, the uterus, suprarenal glands and thyroid gland. Eosinophilic myelocytes often formed more than 50 per cent of the infiltration, and were everywhere conspicuous, in the bone marrow, as well as in the viscera.

The case was regarded as unusual because of:

1. The appearance of atypical cells (monocytoid promyelocytes?) in the blood at the onset of the disease.
2. The presence of hematomas in the spleen with terminal rupture.
3. The discrepancy in character between the cells of the circulating blood and those infiltrating the tissues.
4. The preponderance of eosinophilic myelocytes in the tissues in what would otherwise appear to have been a case of acute myeloid leukemia.

#### DISCUSSION

NATHAN ROSENTHAL: This case of aleukemic leukemia or leukopenic leukemia is most unique. At first one would be inclined to classify it incorrectly as agranulocytic angina, and I was glad to hear that this was not done. Similiar cases have been classified as agranulocytic angina on account of the lack of a postmortem examination. Furthermore, the persistent presence of premature myeloid cells rules out such a condition. The question of whether they were monocytes or myeloblasts is not very important, although it is beginning to be recognized that there is a leukemia of the monocytic type. The predominating cells in monocytic leukemia show a negative oxidase reaction, indicating a premature type of monocyte. I do not know whether an oxidase stain was done on the monocytic cell found at first in the present case.

WILLIAM P. THOMPSON: These abnormal cells were oxidase-positive. There is one other interesting thing in connection with this case. The same day this patient came in, a young man was admitted with an identical clinical picture, a sore throat due to infection with a hemolytic streptococcus and the same blood picture. He got the better of his sore throat, and one week later the blood picture was normal, while that of our patient progressed to the terminal pathologic picture of leukemia. On admission these patients were identical clinically, presenting similar white counts and differential counts, including the presence of these cells that we do not know how to classify.

MAURICE N. RICHTER: I have had the opportunity of studying blood smears and sections in a number of cases diagnosed as monocytic leukemia, and I have been impressed by two things: one is that it is almost always possible to find transitional forms between the myeloblasts and the monocytes, and the other is that it is exceedingly difficult in sections to be sure that the monocytes are coming from reticulo-endothelial cells, as is generally reported. For these reasons I am a little skeptical as to the extent to which monocytic leukemia can be regarded as an entity. In regard to this particular case, I do not think now that the question of monocytic leukemia enters. This was only an abnormal feature of the blood. From the histologic standpoint it is purely a myeloid leukemia.

ANGELO M. SALA: We heard Dr. Sproul say that there was a streptococcus blood culture. Was any significance attached to that?

WILLIAM P. THOMPSON: No, just one flask showed a positive culture; the others did not. The throat cultures several times gave an almost pure growth of the hemolytic streptococcus.

NATHAN ROSENTHAL: With regard to monocytic leukemia, there have been more cases reported lately. I recently observed a case in which the blood cells were studied by the supravital stain, following the technic of Sabin. The typical rosette was present in most of the cells.

MAURICE RICHTER: What was the origin of the monocytes?

NATHAN ROSENTHAL: The cells are derived from the reticulo-endothelial system.



## Book Reviews

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**Pathologie und Klinik in Einzeldarstellungen.** Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band V: Mass und Zahl in der Pathologie. Von Professor Dr. Robert Roessle, Direktor des Pathologischen Instituts der Universität Berlin, und Dr. Frédéric Roulet, Oberarzt am Pathologischen Institut der Universität Berlin. Paper. Price, 16 marks. Pp. 144, with 27 illustrations. Berlin: Julius Springer, 1932.

This book consists of a series of monographs, of which the following have been published: "Der appendicitische Anfall" by Aschoff; "Gewebsproliferation und Säurebasengleichgewicht" by R. Bálint and S. Weiss of Budapest; "Die Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie" by J. Erdheim; "Thrombose" by A. Dietrich of Tübingen, and now "Mass und Zahl" by Roessle and Roulet. Roessle said that he was pleased to undertake the task proposed to him, of contributing to the series, because it naturally brought about an assay of the measurements he had made at postmortem examinations during many years, and he included with his own those made by William Müller, his predecessor at Jena. The opportunity was presented of determining the value of such estimates, of passing on to others information useful to them in their own work and of filling a real gap in anthropometry of human viscera.

The suggestion for a monograph on "body weight, organ weight and the caliber of vessels" undoubtedly had its birth in the numerous investigations completed by Roessle and his assistants during the past decade or two: studies of the growth and age of large arteries and their relation to disease of the blood vessels; the pathology of body growth; the pathology of growth in children; growth of the internal organs in children; characteristics of mortality in Basel, a contribution to geographic pathology, and his "Technic der Obduction mit Einschluss der Massmethode" in the "Abderhalden Handbuch." Pathologists are not in accord as to the importance of accurate measurements as a routine in postmortem examinations, although their desirability in medicolegal inquiries or when the bodies are immature is generally admitted. Roessle believes that they are an aid to scientific diagnosis and play a disciplinary rôle, and that they are useful to check the accuracy of anatomic diagnoses and to detect inconsistencies in protocols. In discussing methods, he reviews many sources of error, such as the escape of blood from the liver and lungs and the mistake of including hilar tissues, subepicardial fat and clots of blood in the heart with organ weights. Terminal edema of the lungs and fluids in them from gravity have so interfered, that it is doubtful whether the weight of normal lungs is known. Some of these details and others bearing on the same topic are mentioned in his account of postmortem technic.

This volume is a profusion of statistics in tables like those in that indispensable and time-honored thesaurus, Vierordt's "Daten und Tabellen"; indeed, as an expansion of the familiar standards of the older work, those assembled by Roessle and Roulet possess an importance of the first order. In Vierordt's work, it will be recalled, there is no discussion of the values displayed in tables. In "Mass und Zahl," however, tables are an appendage to text, and personal observations are included and contrasted with determinations made by other investigators and reported, for the most part, since the last edition of "Daten und Tabellen" in 1906. The entire range, prenatal and postnatal, of human life is covered. Although there are no tables devoted solely to weights of organs in various types of disease, a few are wholly concerned with "organ correlation." The weight of the heart is compared with body length, body weight and the weight of some viscera and of a biceps muscle. In another table, weights of the liver, spleen, pancreas and body are correlated; in a third, those of the testes and suprarenal glands.

Physical anthropology has been overwhelmingly occupied with the exterior of the body and with the skeleton so far as it contributes to shape. Knowledge of the viscera of the separate human races is in its infancy. The information Roessle has contributed was from his work at Jena in middle Germany, with the agrarian people or workmen of the better class, and from that at Basel, with its proletarian and cosmopolitan inhabitants; other estimates were a fruit of the World War. Investigations by Americans are included in the several concluding pages of bibliography: Scammon, Mall, Wilder, Moon, A. W. Meyer, Benedict and Talbot. Measurements by some of them are included in the tables. The usual list of contents follows the preface, but unfortunately there is no index. The last table contains weights in bodies selected because the form of death promised normal weights: eighty-seven bodies of males and twenty-eight of females, with practically all ages represented. Evidently a prolonged overhauling of records was required to make the selection, because the period during which the bodies were examined covered thirteen years. One of the interesting conclusions is that in no single body is there what might be termed an ideal average for all the computations, weights of important organs, body weight and length, trunk circumferences and the like. The authors hope that perhaps the data offered may stimulate others to furnish measurements so that ultimately indexes and ratios shall be available pointing definitely to types of constitution or of disposition to particular forms of disease. They look forward to a time when records shall be so abundant that "gland formulas" may be employed to explain why in some bodies of persons killed outright wide variations from average weights, volume and the like are present, notwithstanding that there is no evidence of disease, and they suggest that what they have presented is a step in that direction.

## Books Received

RECENT ADVANCES IN PATHOLOGY. By Geoffrey Hadfield, M.D., F.R.C.P. (Lond.), Professor of Pathology in the University of London, Pathologist to the Royal Free Hospital, and Lawrence P. Garrod, M.A., M.B., B.Ch. (Camb.), M.R.C.P. (Lond.), Bacteriologist and Lecturer in Bacteriology, Late Demonstrator of Pathology, St. Bartholomew's Hospital. Price, \$3.50. Pp. 392, with 67 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF HEARING: I. STUDIES IN THE LOCALIZATION OF SOUND. A. THE LOCALIZATION OF SOUNDS IN THE MEDIAN PLANE. By J. H. Shaxby and F. H. Gage. B. SOME FACTORS IN AUDITORY LOCALIZATION. By H. E. O. James and Marion E. Massey. Medical Research Council, Special Report Series No. 166. Price, 1 shilling, net. Pp. 51. London: His Majesty's Stationery Office, 1932.

ALCOHOL AND INHERITANCE: AN EXPERIMENTAL STUDY. By F. M. Durham and H. M. Woods, Medical Research Council, Special Report Series No. 168. Price, 1 shilling, 3 pence, net. London: His Majesty's Stationery Office, 1932.

IMMUNITÄT, ALLERGIE UND INFektionsKRANKHEITEN: PRAKTISCHE ERGEBNISSE DER WISSENSCHAFTLICHEN FORSCHUNG UND KLINISCHEN ERFAHRUNG. Herausgegeben von Rudolf Degkwitz, Erich Leschke, Hans S. Rosenthaler, Georg Schröder und W. Storm van Leeuwen. Schriftleitung; E. Michelsson. Band III, Heft 4-6. Sonderheft. Grundfragen der Immunbiologie und Allergielehre. Price. paper. 7.50 marks. Pp. 133-253. Munich: Verlag der Ärztlichen Rundschau Otto Gmelin, 1932.

## THE HYALINE MEMBRANE IN THE LUNGS

### I. A DESCRIPTIVE STUDY

SIDNEY FARBER, M.D.

AND

JAMES L. WILSON, M.D.

BOSTON

A peculiar eosin-staining membrane—the so-called hyaline membrane—lining the walls of bronchioles and alveoli was noted in the lungs of patients who died of influenza in the epidemic of 1918. This formation was so frequently seen that it was considered pathognomonic of the disease. Recently, we have seen the same pathologic picture under such a variety of circumstances that it seems advisable to reconsider the subject.

### GENERAL CONSIDERATIONS

The acute, or “fulminating,” type of influenzal pneumonia was one of the most interesting manifestations of the disease as seen in the last epidemic, and it attracted the attention of a number of American workers. Winternitz<sup>1</sup> noted an average duration of nine days for this form of pneumonia. According to other observers, it varied in duration from three to twenty days. The lungs, as seen at postmortem examination, were airless and distended with a bloody, serous fluid. The pleural surfaces were mottled with brilliant colors. Occasional large areas of hemorrhage suggested infarcts. Emphysema was often a striking feature, extending into the mediastinal tissues. It was particularly in this type of pneumonia that the hyaline membrane was noted.

As the observations were summarized by Opie<sup>2</sup> in a recent review, this hyaline material may form a continuous layer resting on the walls of the alveolar ducts and extending across the orifices of the tributary alveoli, and may be found similarly placed within alveolar sacs (fig. 1).

It is interesting to note that although the hyaline membrane was described by a number of American workers after the last epidemic, no description of it can be found in the German literature dealing with the epidemic. In an excellent recent review of the German literature by

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From the Department of Pathology and the Department of Pediatrics of the Harvard Medical School, and of the Infants' Hospital and the Children's Hospital.

1. Winternitz, M. C.; Wason, I. M., and McNamara, F. P.: *The Pathology of Influenza*, New Haven, Conn., Yale University Press, 1920.

2. Opie, E. L.: *Arch. Path.* 5:284, 1928.

Luksch,<sup>3</sup> description of such a histologic picture is conspicuously lacking. Pseudomembranes composed of purulent exudate applied to the mucosal surface of the larynx, trachea and bronchi in cases of influenza were described by Meyer,<sup>4</sup> Stettner<sup>5</sup> and others, but the descriptions do not apply to the hyaline membranes seen in this country.

In the American literature dealing with the hyaline membrane there is a marked lack of agreement in regard to the significance of the picture, the composition of the membrane and the mechanism of its

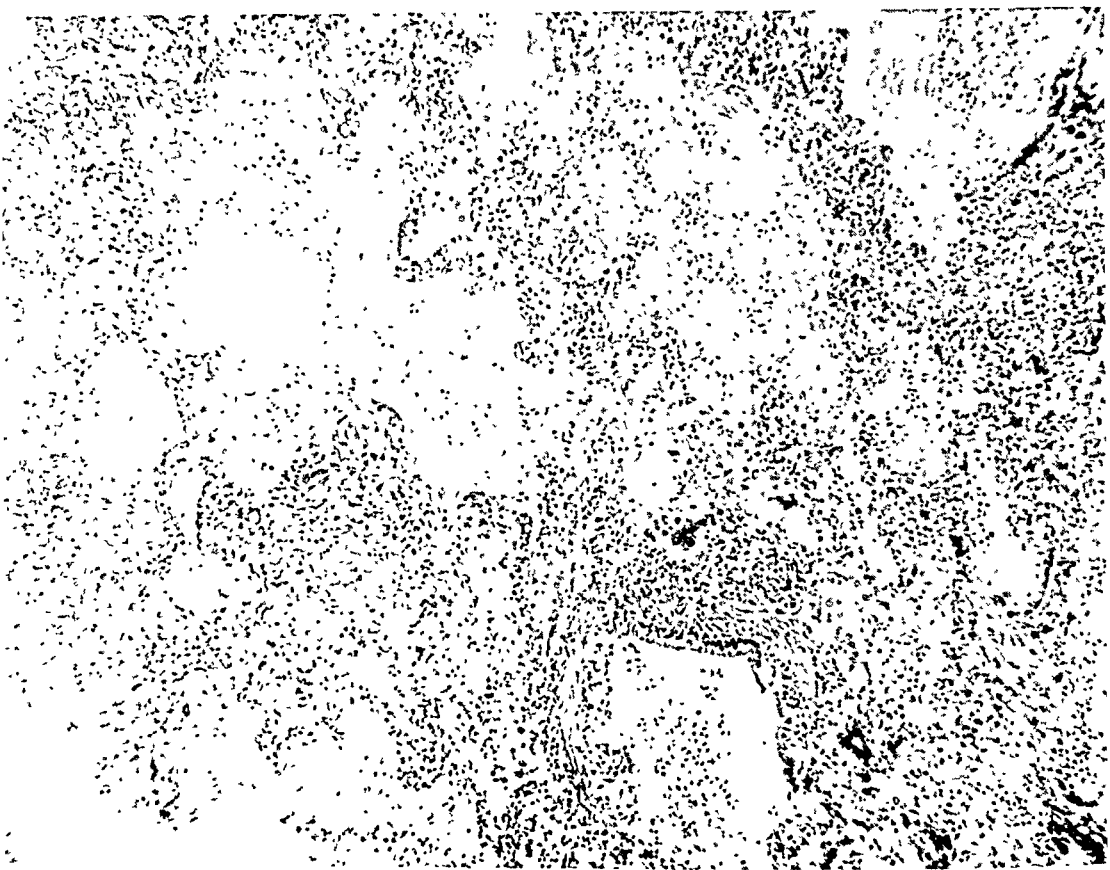


Fig. 1.—Photomicrograph of lung from an adult who died of acute influenzal bronchopneumonia. Note the eosin-staining hyaline membranes. Hematoxylin and eosin; reduced from a magnification of  $\times 112$ .

formation. It has been variously described as a fibrin or a fibrinoid deposit, the "lesion of characterization" of influenza, and the "virus lesion" distinct from the changes due to secondary pathogenic invaders.

The various explanations regarding the mechanism of the formation of the hyaline membrane can be divided into three main groups: (1)

3. Luksch, Franz: *Arch. Path.* 5:448, 1928.

4. Meyer, S.: *Deutsche med. Wchnschr.* 45:38, 1919.

5. Stettner, E.: *München. med. Wchnschr.* 32:872, 1918.

that in which the membrane is regarded as the result of hyalinization of necrotic alveolar walls, (2) that in which it is considered to be the result of injury to the capillary bed and (3) that in which it is held to be the result of air forced into exudate.

Winternitz and his associates, in a series of communications,<sup>6</sup> attributed the membrane to hyalinization of the necrotic material resulting from the action of some powerful agent on the alveolar walls. They described the membrane as a swollen, homogeneously staining material, quite without architecture, replacing the alveolar epithelium. They stressed the generalized congestion, the hemorrhage into the pulmonary parenchyma and the albuminous, rich, serous exudate that occupies the foreground in the picture of the acute disease. These features were observed in patients who had suffered from intense cyanosis, dyspnea and pulmonary hemorrhage. The acute necrosis, which involves first the epithelium of the trachea, bronchi and bronchioles, and which may extend beyond the epithelium into the walls of these structures or may even destroy *en masse* the walls of the alveoli, causes a lesion which they believed occurred characteristically in influenza and could not be brought about by other types of acute pulmonary infection. They did believe that the action of various war gases on the lungs produced effects similar to those seen in influenza. Similar changes after inhalation of war gases were noted by a number of German workers, as is shown in the monograph of Kuczynski and Wolff.<sup>7</sup> Aschoff<sup>8</sup> also described the appearance of the alveolar walls after inhalation of war gas and spoke of swelling (imbibition) and homogenizing of the alveolar walls due to chemical toxic changes.

Brannon and Goodpasture,<sup>9</sup> in 1924, suggested the possibility that the hyaline membrane was the result of damage caused by a circulating toxin which occurred in cases of severe influenza, and which caused mild injury of variable degree to the capillary bed of the lungs. They believed that this injury to the pulmonary capillaries permitted an exudation into the alveolar spaces of fibrinogen-containing serum, and that from this exudate fibrin would quickly form, adhere to the alveolar walls, and become fused, thereby forming a "membrane."

Wolbach,<sup>10</sup> and later Opie, Blake, Small and Rivers,<sup>11</sup> offered the suggestion that the hyaline membrane was related to the action of air on some of the body fluids or exudates. Wolbach described the membrane as being closely applied to the walls of the alveoli and alveolar ducts, and as sometimes apparently replacing them, but in other areas as being actually separated from these walls by desquamating epithelial cells or by red cells and leukocytes. He believed that the formation of the membrane was not dependent on the presence of necrotic alveolar walls. Wolbach concluded that the arrangement and outline of this hyaline material were determined by its contact with air, which must be under some tension.

6. Winternitz, M. C.: *The Pathology of War Gas Poisoning*, New Haven, Conn., Yale University Press, 1920. Winternitz.<sup>1</sup> Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: *J. Exper. Med.* **32**:199, 205 and 211, 1920.

7. Kuczynski, M. H., and Wolff, E. K.: *Ergebn. d. allg. Path. u. path. Anat.* **19**:947, 1921.

8. Aschoff, L., quoted by Kuczynski and Wolff.<sup>7</sup>

9. Brannon, D., and Goodpasture, E. W.: *Arch. Int. Med.* **34**:739, 1924.

10. (a) Wolbach, S. B.: *Bull. Johns Hopkins Hosp.* **30**:104, 1919. (b) Wolbach, S. B., and Frothingham, C.: *Arch. Int. Med.* **32**:571, 1923.

11. Opie, E. L.; Blake, F. S.; Small, J. C., and Rivers, T. M.: *Epidemic Respiratory Disease, the Pneumonias and Other Infections of the Respiratory Tract Accompanying Influenza and Measles*, St. Louis, C. V. Mosby Company, 1921.

## MATERIAL AND PROBLEMS

A number of pertinent questions are raised by this lack of agreement in the literature regarding the histogenesis of the membrane. These questions shape themselves as follows:

1. Is the hyaline membrane pathognomonic of influenzal pneumonia? Is it the "virus lesion" of influenza?
2. What is the composition of the membrane?
3. What is the mechanism of its formation?

## SPECIFICITY OF THE HYALINE MEMBRANE FOR INFLUENZA

The hyaline membrane has been generally regarded as pathognomonic of influenza, and it has been described as the distinctive lesion caused by the virus of influenza. In 1924, Brannon and Goodpasture<sup>9</sup> reported two instances of the occurrence of hyaline membranes in the absence of any association with influenza. Johnson,<sup>12</sup> in 1923, quoted a personal communication from Professor Hayashi of Japan to the effect that hyaline membranes were observed in pneumonic plague.

In a recent communication, Farber and Sweet<sup>13</sup> called particular attention to the occurrence of similar membranes in the lungs of new-born infants with a birth history suggesting intra-uterine asphyxia with aspiration of amniotic sac contents. The term vernix membrane was applied to these structures. These membranes are indistinguishable in appearance, position and ordinary staining reactions from the hyaline membranes of influenza. Since our last report, we have observed vernix membranes in the lungs of over fifty new-born infants.

During the past two years, we have seen typical hyaline membranes in the lungs of twenty infants and young children, varying in age from 3 months to 4 years, whose deaths were caused by pneumonia due to infection with *Streptococcus hemolyticus*. In addition, hyaline membranes were observed in the lungs of a young child who died of acute tuberculous pneumonia, a young adult who died of streptococcic bronchopneumonia, and a man of 42 whose illness ran the typical course of lobar pneumonia. In the last case, numerous membranes were found in a less involved portion of the lung (figs. 2 and 3).

The membranes in all of these instances were identical in appearance and staining reactions with those seen in influenzal pneumonia. There was no clinical or pathologic evidence of influenza in any of these cases.

It is evident from the occurrence of typical hyaline membranes in such a variety of conditions in the absence of any suggestion of influenza

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12. Johnson, W. C.: *Proc. New York Path. Soc.* **23**:138, 1923.

13. Farber, S., and Sweet, L. K.: *Am. J. Dis. Child.* **42**:1372, 1931.

that the hyaline membrane is not pathognomonic of influenza. There remains, however, the possibility that the membrane may represent the distinctive lesion of the virus of influenza, a lesion that may be present only incidentally in the various instances that we have cited. This question we shall attempt to answer later in this study.

#### THE COMPOSITION OF THE HYALINE MEMBRANE

The typical membrane consists of a mass of homogeneous amorphous material, in which cellular debris and bacteria can occasionally be found.

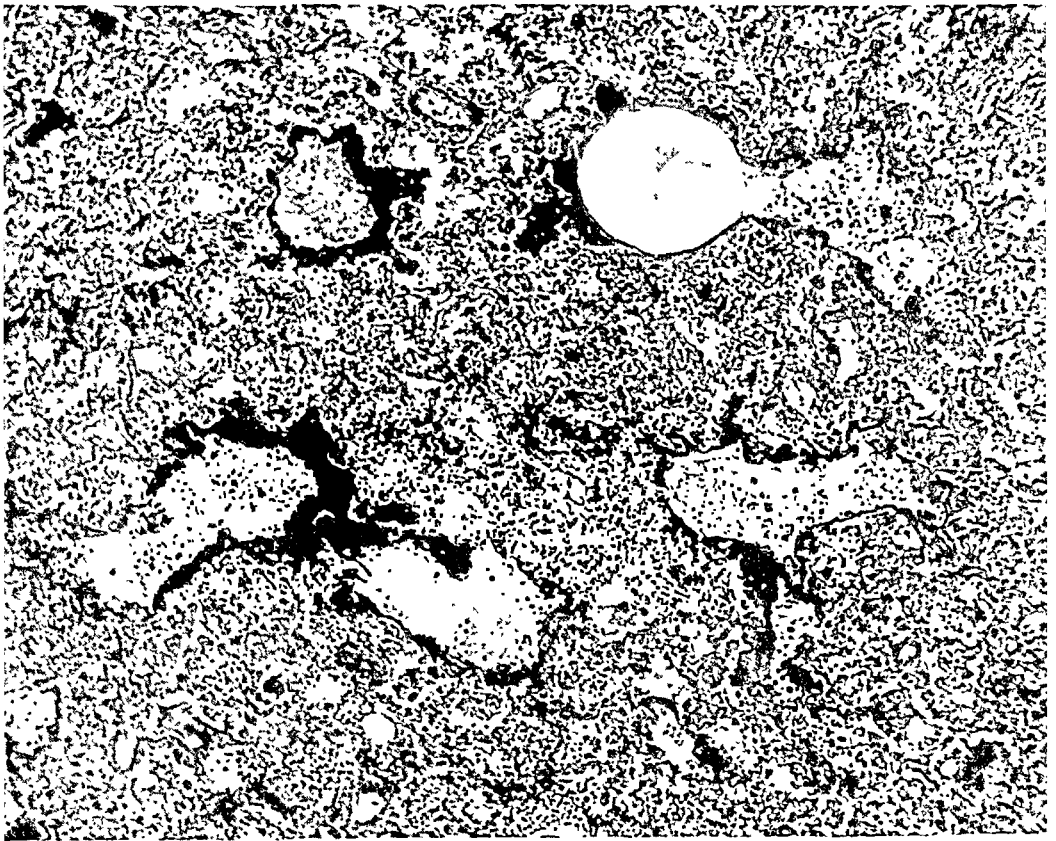


Fig. 2.—Photomicrograph of lung from an infant who died of acute streptococcal bronchopneumonia. Note membranes and bubble of entrapped air. Aniline blue; reduced from a magnification of  $\times 104$ .

Both in cases of influenza and in those of pneumonia that we studied, the appearance of the material in the membrane is that of fused, resolving exudate consisting of necrotic mononuclear cells, leukocytes, erythrocytes, altered fibrin and serum, and debris from the alveolar walls, when necrosis of these walls is present. The material in the membrane can best be compared to the exudate in lobar pneumonia during the stage of resolution when autolysis of the exudate is taking place and cellular



detail can no longer be identified. Under these circumstances, former staining reactions are lost, and the necrotic materials stain deeply with eosin. A similar change characterizes the appearance of necrotic débris in an area of tuberculous caseation. When viable cells are present in the membrane, organization is probably taking place, for, as Wolbach<sup>10</sup> showed, during repair there is invasion of the membrane by leukocytes and phagocytes. Eventually organization with connective tissue takes place in the membrane, as is well shown in the illustration in his paper.

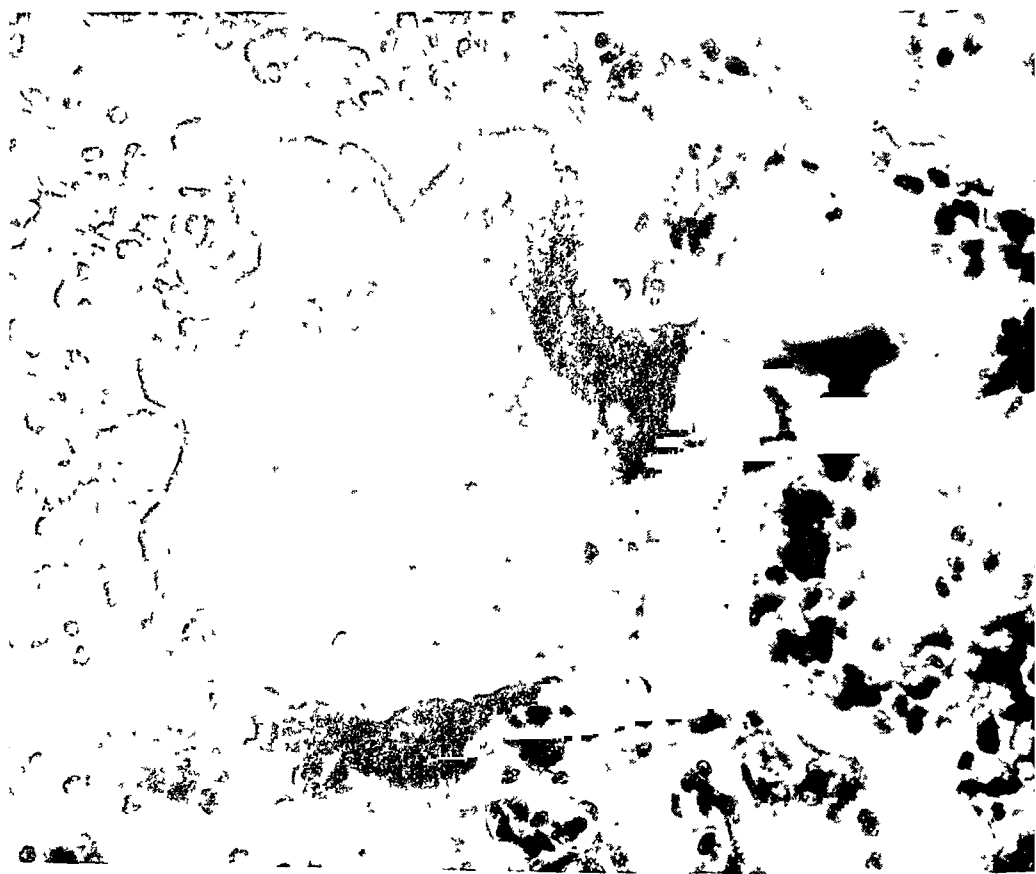


Fig. 3.—Photomicrograph of lung; a high power magnification of a detail of figure 2; reduced from a magnification of  $\times 500$ .

The membrane found in the lungs of new-born infants (vernix membrane) consists of amorphous material, embedded in which can be found cornified epithelial cells. It was shown<sup>13</sup> that this type of membrane, identical in appearance and staining reactions with the hyaline membrane, consists of aspirated amniotic sac contents. The bulk of this membrane is composed of vernix caseosa. There is no evidence of necrosis in association with the vernix membrane.

Differential stains (phosphotungstic acid hematoxylin) failed to show fibrin in any of the membranes in this study. In a few instances, in

the cases of pneumonia, in the presence of a fresh exudate, occasional strands of fibrin could be seen superimposed on, or pressed against, the membrane. The fresh deposits of fibrin could easily be distinguished from the main body of the membrane by the differential stain employed. In alveoli bordering those containing membranes, occasional small networks of fibrin could be identified readily in the phosphotungstic acid hematoxylin stain. However, there is no doubt that altered or digested fibrin may be one of the components of the membrane in the cases of

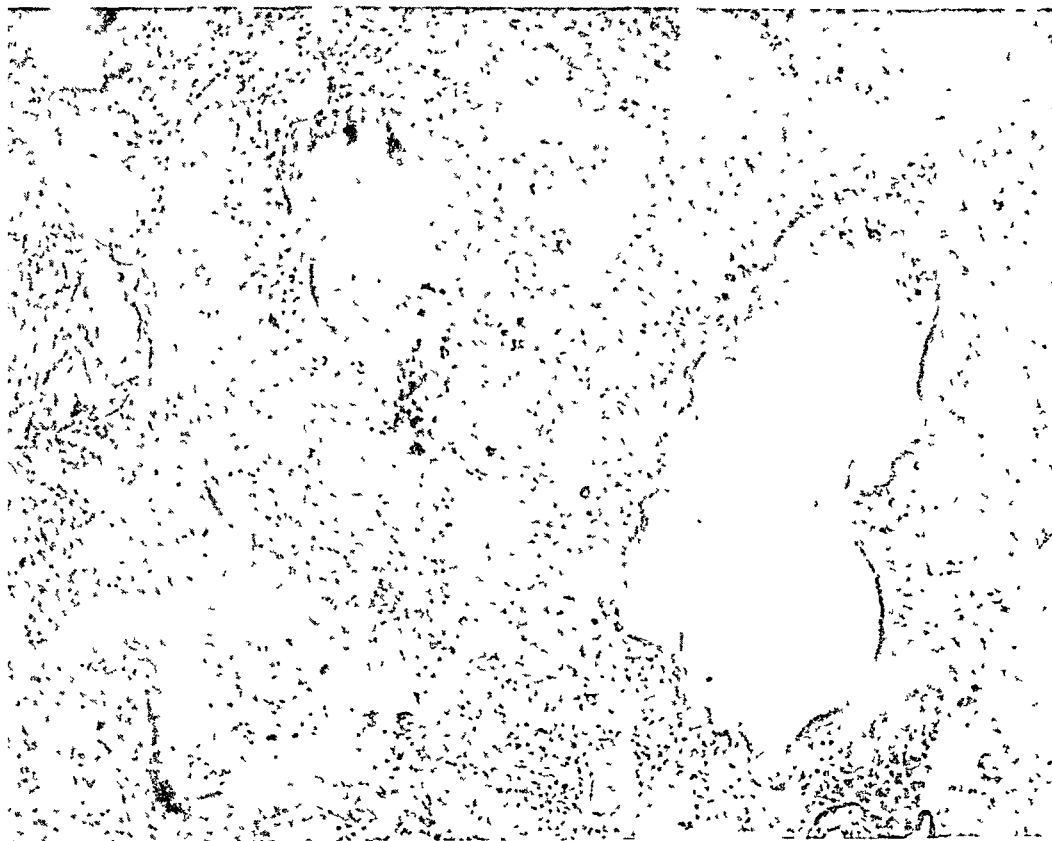


Fig. 4.—Photomicrograph of lung from the same patient as in figure 1. Note the large amounts of fat in the membrane. Scarlet red; reduced from a magnification of  $\times 104$ .

pneumonia, despite the failure of staining reactions, for as Brannon and Goodpasture pointed out, the hyaline fibrin of a diphtheritic exudate does not retain the staining reactions of fibrin.

Scharlach R stains after formaldehyde fixation revealed a surprising finding: In the lungs of an adult who had died of acute, or fulminating, influenzal pneumonia, and in those of the majority of the young children who died of streptococcic pneumonia whom we examined, large amounts of fat were present in the form of both large and fine droplets, which

had coalesced to form broad, continuous, homogeneous bands of red-staining material, duplicating perfectly the outline of the membrane as seen in ordinary stains. Scattered throughout other portions of the sections there could be found small droplets of fat, usually within phagocytes. In other instances, fat stains were not so striking. However, even in cases in which only slight amounts of fat were present, it existed in the form of fine droplets within phagocytes, comparable in amount to that which can be found in any pneumonic lung when a considerable amount of exudate is present (fig. 4).

Fat in large amounts is constantly present in the vernix asphyxial membranes, representing here the high fat content of the aspirated vernix caseosa.

To summarize: The hyaline membrane in influenzal pneumonia, the membranes associated with the various types of pneumonia in our series, and the vernix membranes have the same staining reactions. They vary from pink to red with hematoxylin and eosin, and from salmon pink to red with the phosphotungstic acid hematoxylin stain. There is some variability in the amount of fat in the membranes associated with pneumonia. The vernix membranes can be differentiated from all other hyaline membranes by the cornified epithelial cells from the amniotic sac contents embedded in the vernix membrane. These can usually be identified in the ordinary stains. The Gram stain followed by acid alcohol decolorization has proved of use in doubtful cases. Special stains failed to show unaltered fibrin in the membrane.

#### MECHANISM OF THE FORMATION OF THE MEMBRANE

The membranes that we have studied varied in size and appearance from thin strands to broad, sweeping bands of eosin-staining amorphous material, in which cellular débris was frequently embedded. Often a membrane filled more than half of the lumen of a bronchiole or an alveolar duct, usually becoming thinner as it followed the walls of the finer ramifications of an alveolar duct. Characteristically, it sealed off whole groups of pulmonary structures, most often surrounding a bubble of entrapped air or a collection of serum. In some cases, the membranes were so numerous and their continuity so preserved that an efficient barrier to the passage of air must have been presented terminally.

One of the most frequent observations in the cases of pneumonia was that of masses of amorphous eosin-staining material free in the lumens of bronchioles and alveolar ducts. These masses of amorphous material were similar in appearance and staining reactions to the material in the hyaline membrane. Sometimes they were spread thickly around small bubbles of entrapped air in the centers of alveolar spaces. These masses varied greatly in size and shape. This finding recalls

exactly that of masses of vernix caseosa in similar situations in the lungs of new-born infants.

Typical membranes, formed at some distance from the alveolar walls, and separated from them by serum or red cells, were frequently encountered. Necrosis of alveolar walls was present in a number of the cases of streptococcic pneumonia and in the case of influenzal pneumonia. Although membranes frequently were seen adjacent to necrotic alveolar walls in these cases, we have repeatedly observed membranes lining intact walls with perfect preservation of the epithelium. The vernix membrane occurred most often in cases in which pneumonia was lacking and in which no evidence of necrosis of the alveolar walls could be found.

In the series of pneumonic lungs of various types in which we found typical hyaline membranes, the absence of large amounts of exudate in areas where membranes were numerous was at first striking. In regions where membranes were lacking, the alveoli were usually completely filled with an acute inflammatory exudate consisting of leukocytes, erythrocytes and fibrin in a state of good preservation. The leukocytes in such areas stained clearly, and the exudate appeared recent in origin. Where membranes were numerous, exudate with good cellular detail was absent, except in rare instances when a superimposed acute exudate was found. These different pictures in the same person point to a variation in the age of the inflammatory processes and explain the apparent aplastic appearance of the exudate in areas where membranes were numerous. In such areas, the pathologic process had advanced to a point where autolysis of the exudate was taking place, and cellular detail could no longer be identified. The characteristic eosin-staining appearance and the amorphous, homogeneous consistency of the components of the membrane in the cases of pneumonia depend on the presence of necrotic debris and of old, partially autolysed exudate.

Wolbach <sup>10b</sup> described in two instances typical hyaline membranes surrounding bubbles of air in the areolar tissues of the mediastinum, which was the site of emphysema and also of pneumococcic mediastinitis. On one occasion we observed typical membranes outside the lung, in the mediastinal areolar tissues of a young child who had died of a descending infection of the upper respiratory tract by *S. hemolyticus*. A tracheotomy had been performed, and air had dissected into the mediastinum, which had participated in the streptococcic infection. Lining the emphysematous areas were membranes that had the same appearance and staining reactions as those in the lungs (fig. 5).

The various theories regarding the formation of the membrane have already been outlined. The hypothesis of Brannon and Goodpasture rests on the existence of an injury of the capillary bed with an outflow

of fibrinogen-containing serum. Winternitz and his associates, reporting on their experiments with war gas poisoning and insufflation of hydrochloric acid, described, in addition to the necrosis of the alveolar walls, an albuminous, rich, serous exudate that was prominent in acute disease and thick, colloid-like material within the alveoli. Oberndorfer<sup>14</sup>



Fig. 5.—Photomicrograph of mediastinum showing interstitial emphysema, from a person with streptococcic mediastinitis. Note the space occupied by air lined by typical hyaline membrane. Aniline blue; reduced from a magnification of  $\times 68$ .

emphasized the degree of vascular damage in influenza, describing a primary damage resulting in increased capillary permeability. There is abundant mention of such damage in the literature. According to Luksch,<sup>3</sup> Stoerck and Epstein found changes in the elastica interna and

14. Oberndorfer, S.: München. med. Wchnschr. 65:811, 1918.

muscularis of the medium-sized arteries, with areas of necrosis in the muscularis. Thrombi have been noted by numerous observers and have been regarded by Leichtenstern<sup>15</sup> as primary in a certain number of cases of influenza. The large collections of serum in the lungs and the copious amounts of thin, serosanguineous exudate noted both clinically and pathologically in influenzal pneumonia indicate vascular damage. However, such damage is not peculiar to influenza or to inhalation of war gases. Vascular damage due to a variety of causes will bring about similar pictures, as, for example, in streptococcic pneumonia. Oswald showed (according to Wells<sup>16</sup>) that in inflammation the permeability of the vessels for protein becomes specifically altered, so that not only the less viscous albumin and pseudoglobulin pass through their walls, but also the more viscous euglobulin and fibrinogen. Fluid may collect in alveolar spaces owing to other causes. Increased blood pressure, particularly in the presence of damage to the vascular bed, or obstruction to the lymphatic drainage, either by thrombotic plugging of the lymphatics or by pressure on the outside, may play a part.

Damage to the vascular bed undoubtedly accounts for a great part of the exudate in the alveolar spaces in influenza and also in streptococcic pneumonia. Although this exudate contributes later to the composition of the membrane, it is not in itself an adequate explanation for the actual formation of the membrane.

There remains to be considered the theory that the membrane represents a hyalinization of necrotic alveolar walls, similar to that after war gas poisoning or after insufflation of hydrochloric acid. There are a few objections to this theory. As we have stated, we repeatedly observed typical hyaline membranes lying against healthy alveolar walls, and we also noted membrane formation at some distance from alveolar walls. The vernix membranes are usually found lining structures in which necrosis cannot be demonstrated. That the membrane is not related specifically to degenerated epithelium is shown by the two cases reported by Wolbach and by our own similar case in which typical membranes were found in the presence of emphysema and mediastinitis in the tissues of the mediastinum, where no epithelium existed. Necrosis of the alveolar walls, when present, probably adds to the necrotic material taking part in the composition of the membrane. However, an explanation for the occurrence of the hyaline membrane must be sought elsewhere than in a specific effect of some irritant on the alveolar walls. It is interesting to recall that the vernix membrane was first described as an "influenza-like" lesion, and an attempt was made to relate the

15. Leichtenstern, quoted by Luksch.<sup>3</sup>

16. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

membrane to the aspiration of some irritant, such as lysol, from the vaginal contents during birth. However, a number of our most striking examples of the vernix membrane were found in the lungs of infants who had been delivered by cesarean section where this possibility did not exist.

Any explanation of the formation of the membrane must take into consideration not only the membrane seen in influenzal pneumonia, but also that found in the various types of pneumonia that we have described and the membranes occurring after aspiration of amniotic sac contents. In general, they all have the same appearance and staining reactions, and except for a few details in the composition of the vernix membrane, they are indistinguishable from one another.

The outstanding clinical and pathologic features of these cases of influenzal and streptococcic pneumonia are marked dyspnea (with emphysema) in addition to the presence of a large amount of semifluid material in the air spaces. Similar features characterize the cases of aspiration of amniotic sac contents which we have described. The small and large masses of eosin-staining amorphous material found free in the alveolar spaces both in the persons who died of pneumonia and in the new-born infants whom we studied have been described. These masses we regard as early stages in the formation of the membrane before the material has been pressed against the alveolar walls. From a study of our material, with particular reference to these free masses, we believe that a sequence of events leading to the formation of the membrane can be followed. This sequence can be traced from the coalescence of small masses of amorphous eosin-staining material, free in the alveolar ducts and later in the alveolar spaces, with dispersion toward the periphery to an ultimate close application of the material against the alveolar walls in characteristic membrane formation. This sequence is similar to that noted after aspiration of amniotic sac contents with formation of the vernix membrane.<sup>13</sup> The dispersion of the coalesced amorphous materials to the periphery, the characteristic picture of such substances surrounding bubbles of entrapped air, and the occurrence of typical membranes in the mediastinum in the presence of mediastinal emphysema and exudate all demonstrate a relationship of air to the formation of the membrane—a relationship emphasized by Wolbach<sup>10</sup> and later by Opie, Blake, Small and Rivers.<sup>11</sup>

In the light of our observations it has seemed that the necessary factors for the production of the characteristic hyaline membranes are: (1) the presence of material capable of taking the characteristic eosin stain, (2) air in the alveolar spaces, probably under greater than normal tension, (3) partial obstruction to the passage of the air by semifluid

material in the air passages and (4) dyspnea, which may be interpreted as violent inspiratory efforts in an attempt to force air by this obstruction.

We believe, then, that the position of the eosin-staining substance lying characteristically as a membrane along alveolar walls may be simply the result of the mechanical dispersion of this foreign material toward the periphery by air violently inspired. Further investigation of this mechanical distribution was undertaken.<sup>17</sup>

#### CONCLUSIONS

The hyaline membrane is not pathognomonic of influenzal pneumonia. Identical membranes are found in the lungs of persons with various types of pneumonia and in the lungs of new-born infants after aspiration of amniotic sac contents.

The membrane may contain large amounts of fat. Fibrin cannot be demonstrated in the membrane by special stains.

The formation of the membrane is dependent neither on necrosis of alveolar walls nor on vascular damage, although both these factors may contribute to the production of exudate, which may ultimately enter into the composition of the membrane.

Dyspnea is the outstanding symptom in patients in whose lungs membranes occur.

The position of the eosin-staining substance lying characteristically as a membrane along alveolar walls is simply the result of the mechanical dispersion of foreign material toward the periphery of the air sacs by air violently inspired. This material consists of autolysed exudate and necrotic débris in cases of influenzal and streptococcic pneumonia, and of vernix caseosa and cornified epithelial cells in instances following aspiration of amniotic sac contents.

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17. Farber, S., and Wilson, J. L.: *Arch. Path.*, this issue, page 450.



# THE HYALINE MEMBRANE IN THE LUNGS

## II. AN EXPERIMENTAL STUDY

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In a study<sup>1</sup> of the formation and significance of the hyaline membrane in the lungs, frequently described in influenzal pneumonia, and considered by many pathognomonic of that disease, we concluded that the membrane was not specific for influenza, but was probably produced by the mechanical forcing of air into alveoli through material which took the eosin stain and which was composed either of necrotic débris and autolysed exudate or of aspirated amniotic sac contents. The membrane formation we considered to be a result of foreign material being pressed against the alveolar walls by entrapped air, but having no essential causal relationship to the alveolar walls.

Since the staining reaction was explained by the character of the foreign material in the air spaces, which was not necessarily essential to the formation of the membrane, attention could be centered on the physical mechanism involved. It was determined, therefore, to ascertain in other instances in which similar mechanical conditions in the air passages obtained, whether there were comparable membrane-like formations lining the alveolar walls, not necessarily with the same staining reaction as the typical hyaline membrane. An effort was also made to reproduce experimentally the formation of such membranes, and to ascertain the origin of the fat frequently found in the hyaline membrane.

A consistent clinical feature of patients in whose lungs the characteristic hyaline membrane was found was marked dyspnea, apparently resulting from the necessity of a forceful inspiration of air by and through obstructing exudate. There were frequently such large amounts of fluid in the air passages of patients dying of influenzal pneumonia that Wolbach<sup>2</sup> remarked: "The patient is virtually blow-

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From the Department of Pathology and the Department of Pediatrics of the Harvard Medical School and of the Infants' Hospital and the Children's Hospital.

1. Farber, Sidney, and Wilson, J. L.: *Arch. Path.*, this issue, page 437.

2. Wolbach, S. B.: *Bull. Johns Hopkins Hosp.* **30**:104, 1919.

ing bubbles in his own lung." We have seen similar situations in the following conditions:

1. Poliomyelitis. In many cases of poliomyelitis with respiratory paralysis or with pharyngeal paralysis, the filling of the lungs with exudate or with aspirated mucus and vomitus is common. We have studied the lungs of six such patients, who died while artificial respiration was being maintained in a Drinker respirator. It was understandable that in a final effort to save their lives these patients were subjected to particularly forceful inspiratory efforts produced by the machine—in most cases, a procedure maintained probably for several minutes after the heart had stopped. Under such conditions, air was forced into alveoli filled with variable quantities of serum or foreign materials. In patients who were similarly affected and who were not treated in the Drinker respirator, a similar result but to a lesser extent was brought about by the patient's own efforts at respiration. At autopsy, the lungs were heavy and voluminous and contained large quantities of serum. Microscopically, the alveoli, alveolar ducts and bronchioles were distended and filled with serum. Numerous instances were observed of large air bubbles surrounded by serum which had been pressed against alveolar walls in membrane formation. Often artificial alveolar boundaries were thus formed, and it was sometimes difficult to recognize the true alveolar walls. This always occurred in greatly distended air spaces, and the appearance of large air bubbles entrapped in the serum which had been pressed against the alveolar walls was striking. This picture was not found in areas where consolidation had taken place (fig. 1).

2. Streptococcic bronchopneumonia. An infant, 3 months of age, died following acute streptococcic bronchopneumonia. A pulmotor had been applied by an "emergency crew" when the child apparently was dead. At autopsy, the lungs were tremendously distended, and the alveoli were filled with serum and red cells. A picture similar to that seen in the cases of poliomyelitis was observed. Membranes composed of serum and surrounding entrapped air lined the walls of the greatly distended spaces. In other areas, the alveoli were packed with large amounts of serum and small numbers of red cells (fig. 2).

3. Influenzal pneumonia. An identical picture was seen in a case of influenzal pneumonia in an adult. In a portion of the lung which was filled with serum, again membrane-like formations composed of serum could be frequently found lining the alveolar walls and surrounding bubbles of entrapped air.

4. Lobar pneumonia due to Friedländer's bacillus. Membranes surrounding bubbles of air were noted in the lungs of an adult who had died of Friedländer's type of lobar pneumonia. In the more emphysematous portions of the lung, numerous membranes composed of serum and occasional leukocytes could be found. Frequently such membrane formation was noted some distance from alveolar walls, and occasionally three or more small membranes surrounding bubbles of air could be found in one distended alveolar duct.

5. Acute infection of the upper respiratory tract. In the lungs of five infants who had died of an acute infection of the upper respiratory tract with *Streptococcus hemolyticus*, membranes composed of serum, red cells and sometimes large numbers of leukocytes could be found lining the walls of air spaces and, in a few instances, also pressed against the mucosal surfaces of bronchioles. On a few occasions, in these cases, membranes composed of a loose cellular exudate, poor in fibrin, were observed.

6. Bronchial obstruction. An infant died following partial plugging of the bronchi with aspirated milk. There was partial obstruction to the passage of air,

and violent dyspnea terminating in asphyxia occurred. Microscopically, the essential features were overdistention of the air spaces, the presence of serum in the alveoli, and the formation of membranes composed of serum and a few red cells and applied to the alveolar walls. As was shown by fat stains, little milk had found its way into the alveoli.

7. Cardiac failure. We have observed similar formations in the lungs of a number of patients who had died of cardiac failure after chronic passive congestion of the lungs and increasing dypnea. Again the picture described was noted in numerous areas of the lungs. Membranes composed of serum and occasional red

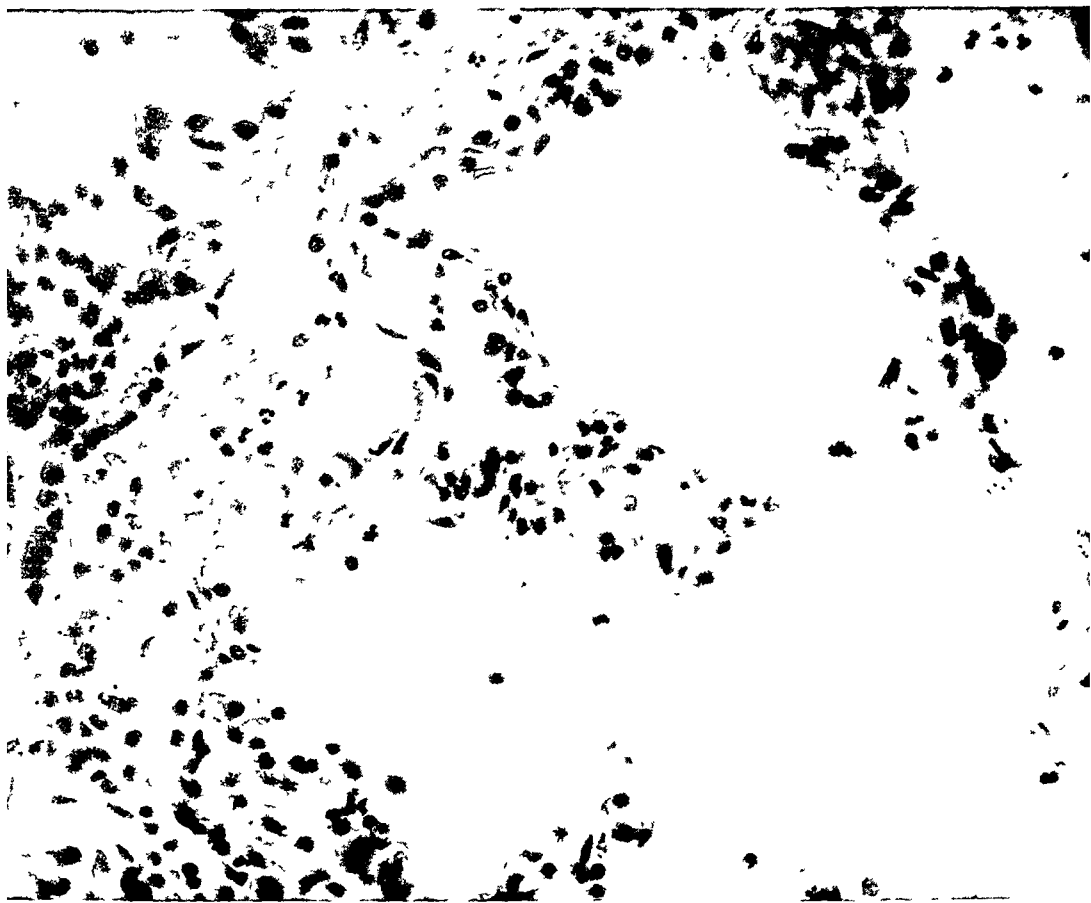


Fig. 1.—Photomicrograph of lung from a young child who died of poliomyelitis. Note the distention of the air spaces and the large amounts of serum in membrane formation surrounding entrapped bubbles of air. Hematoxylin and eosin;  $\times 400$ .

cells, and usually formed at some distance from alveolar walls, could be found with ease.

In all of these instances, marked dyspnea and forceful inspiratory efforts were prominent clinical features. Air was presumably brought into the lungs under an intra-alveolar pressure relatively greater than normal because of the combination of forceful inspiration and partial obstruction. Obstruction to the passage of air was always present, owing to partial plugging of the bronchi by foreign material. The consequence of air being thus forced into and through various types

of obstructing material was the formation of membranes surrounding bubbles of entrapped air. The membranes were formed either against or at some distance from the alveolar walls. The staining reaction of the membrane in each case depended on the substances present in the air spaces, serum, red cells, leukocytes, etc. These membranes were identical in shape, position and mechanism of formation with the vernix membrane and the typical hyaline membrane of influenzal and streptococcic pneumonia.

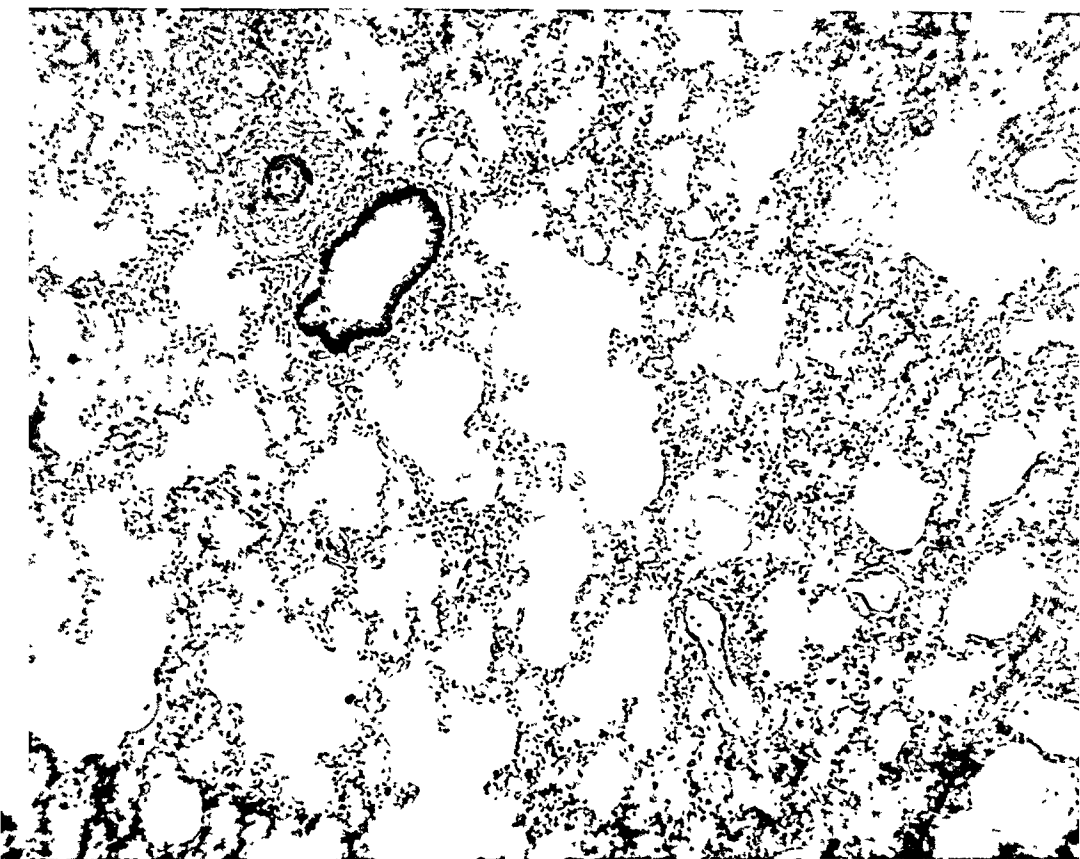


Fig. 2.—Photomicrograph of lung from a 3 months old infant who died of acute streptococcic pneumonia. A pulmotor was applied in an attempt to prolong the infant's life. Note the distended air spaces and the collections of serum in membrane formation surrounding the entrapped air. Hemaxtoylin and eosin; reduced from a magnification of  $\times 112$ .

#### EXPERIMENTAL PRODUCTION OF MEMBRANES

As has been mentioned before, the two factors that seem necessary for the formation of these membrane-like structures in the alveoli are the presence of foreign fluid matter in the air passages and dyspnea with forceful inspiratory efforts. Reproduction of these conditions experimentally without the production of bacterial inflammation was

undertaken in an effort to demonstrate the mechanical basis for this histologic picture. All animals used in these experiments were anesthetized by intraperitoneal injections of either amytal or paraldehyde. The experimental procedures were as follows:

*Asphyxia With and Without Dyspnea.*—A series of rabbits was placed in small, air-tight glass containers and allowed to remain until death from asphyxia occurred. The jars were of such a size that two and one-half hours elapsed before the oxygen was sufficiently used up to produce death. Marked dyspnea resulting from the accumulation of carbon dioxide was present for much of the time.

A second series of animals was allowed to die in a similar manner, except for the constant removal of the carbon dioxide by soda lime. In these animals there occurred only a small amount of terminal dyspnea. At autopsy, the lungs of the first series of rabbits, which died with evidence of marked dyspnea, were dark salmon pink externally. On section, the cut surfaces showed marked congestion, and large amounts of blood flowed freely from the larger vessels. Serum could be readily expressed from the parenchyma on pressure. Microscopically, the blood vessels showed marked congestion, and the alveoli were definitely distended and contained varying amounts of serum, often pressed against alveolar walls in membrane-like formation. The lungs of the second group of rabbits, which died without marked dyspnea, showed no distention of the alveolar spaces and only small amounts of serum in these spaces. No membrane formation had occurred.

*Aspiration of Ink with Resulting Dyspnea.*—A number of young rats were immersed in a solution of 10 per cent india ink for from one to two minutes. They were then removed and permitted to breath with marked dyspnea for a few minutes before they were killed. At autopsy, the ink could be seen distributed throughout all lobes of both lungs. Microscopically, the alveoli contained large amounts of the ink solution, sometimes gathered in small patches without definite shape, free in the air spaces, but more often forming complete rings around air spaces, lining the walls and often sealing off groups of alveoli. These membrane-like formations were usually not very wide and were characteristically formed around entrapped bubbles of air. The india ink membranes were an exact duplication of the hyaline membrane of influenza in shape, position and general outline, and differed only in staining reaction (fig. 3).

*Aspiration of Foreign Material During Artificial Respiration.*—In order to produce even more excessive respiratory movements in the presence of obstructing fluid matter in the air passages than an animal would produce by itself, a small improvised Drinker respirator was utilized. The animals were placed in the respirator after anesthesia and subjected to violent artificial respiration (vacuum up to 30 cm. of water pressure). While artificial respiration was maintained, horse serum, india ink and various types of purulent material were injected into the tracheas of these animals. Respirations were maintained in the Drinker apparatus for varying intervals of time, and then the animals were killed. Cats, rabbits and puppies were used in these experiments. In every case, the material, could be found in the alveolar spaces. The foreign matter was usually all lobes of both lungs. Usually collections of serum, in addition to the injected material, could be found in the alveolar spaces. The foreign matter was usually widely dispersed throughout all the alveoli, sometimes collecting in small patches, but more often forming typical membrane-like structures against the alveolar walls. The most striking pictures were obtained after the injection of india ink, because

of the outstanding color of the ink. However, in all cases, the the same type of formation as regards shape and position could be found.

*Insufflation of Hydrochloric Acid, with Vascular Damage and Dyspnea.*—Several rabbits were subjected to insufflation of hydrochloric acid in imitation of the experiments of Winternitz and his co-workers;<sup>3</sup> 7 cc. of a 1 per cent solution of concentrated hydrochloric acid in physiologic solution of sodium chloride was injected intratracheally. Immediately, the respirations became of a noisy, obstructive type, and diffuse fine râles could be heard over the lungs with the stethoscope. Marked dyspnea was brought about. Frothy, serosanguineous fluid flowed from

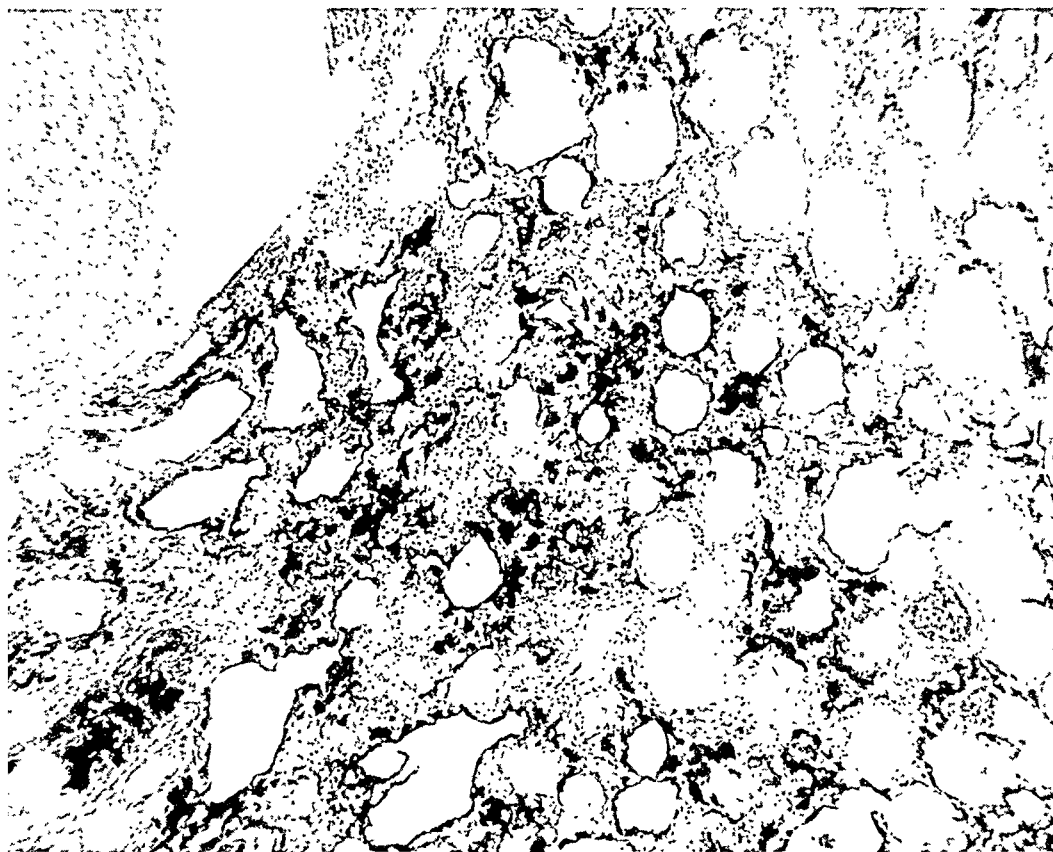


Fig. 3.—Photomicrograph of lung from a young rat that had been immersed in india ink. Note the membranes composed of ink outlining the air spaces and surrounding the entrapped air. Hematoxylin and eosin; reduced from a magnification of  $\times 104$ .

the mouth during the last few minutes of life. At autopsy, the mucosal surface of the trachea was reddened and covered by foamy, serosanguineous fluid exudate. The lungs showed patches of consolidation, and numerous hemorrhagic areas of varying size and shape could be seen beneath the pleura. On section, the cut surfaces were edematous and hemorrhagic. Microscopically, the main features were edema and hemorrhage into the alveoli. The alveolar spaces were filled with large

3. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. 32:199, 1920.

amounts of serum and smaller amounts of red cells. The blood vessels showed marked congestion and early thrombi. In scattered areas, serum was packed against the alveolar walls in membrane formation. In two areas, membranes reminiscent of the hyaline membrane of influenza were seen lining alveolar walls, which showed early necrosis. The membranes consisted of loose fibrin, serum and a small amount of necrotic débris. Distention of the alveolar spaces was a marked feature. The vascular lesions and the marked amounts of serum and blood in the alveoli overshadowed the comparatively small amount of necrosis of alveolar walls in animals treated by insufflation of hydrochloric acid.

*Experiments Showing Exudate as an Essential Factor.*—Several experiments were devised to show the necessity for the presence of an exudate as well as of air under some pressure in order to produce the membrane. A cat was over-ventilated to such a degree that mediastinal emphysema resulted. However, no exudate was present in the mediastinum, and on section, although mediastinal emphysema was demonstrated, there was no membrane formation. In contrast to this situation, air and purulent material obtained from other sources were both forced into the areolar tissues beneath the skin of a rabbit. On section, the air bubbles were surrounded by the fibrinopurulent exudate that had been injected. Typical membrane-like formations occurred in this case, of characteristic hyaline appearance.

*Experiment Further Demonstrating Mechanical Nature of Formation of Membrane.*—To ascertain whether the living animal was necessary for the formation of the membrane, two rabbits were killed, and india ink was injected intratracheally when the animals were in the Drinker respirator. Artificial respiration under increased pressure was then instituted. Microscopically, a picture just as striking as that seen after insufflation of india ink in the living animal was found. Ink membranes in characteristic position and outline could be found in large numbers throughout the lungs.

#### SOURCE OF FAT IN MEMBRANES

A number of experiments were designed to ascertain, if possible, the origin of the fat found in the membranes associated with certain of the cases of influenzal and streptococcic pneumonia. Two possibilities presented themselves. The fat might come directly from the blood stream into the alveolar spaces and so be incorporated in the membrane, or, as the result of autolysis, fat might be liberated from the exudate.

*Experiment to Determine Whether Fat Could Be Drawn from the Blood Stream Under Varying Conditions.*—Puppies, rabbits and rats were placed in the Drinker respirator and subjected to prolonged overventilation with varying degrees of pressure. In all cases there occurred marked distention of alveolar ducts and spaces with occasional rupture of alveolar walls. Serum and small collections of red cells, usually pressed against the walls of the air spaces, could be found. Fat stains (scharlach R after formaldehyde fixation) failed to reveal the presence of fat in the air spaces in any of the animals treated in this manner.

*Experiment to Ascertain Whether Fat Could Be Brought from the Blood Stream into the Alveolar Spaces When the Blood Was Rich in Fat.*—Four cats were fed heavy cream at varying intervals with the hope of temporarily increasing the fat content of the blood stream. Procedures similar to those just described were then carried out, following which the animals were killed. The lungs of these

cats showed emphysema, overdistention of the air spaces and small amounts of serum in the alveoli, usually pressed against alveolar walls. Globules of fat were present in the blood vessels of the lungs or in the peribronchiolar lymphatics, greatly in excess of the amounts usually seen. However, no fat could be demonstrated in the alveolar spaces.

*Other Experiments in Which the Source of Fat Was Studied.*—In the experiments in which horse serum was injected into the trachea and the animal then subjected to violent respiratory movement, no fat could be demonstrated in the resulting membranes. Staining for fat was done on the lungs of all the animals subjected to any of the experimental procedures mentioned in foregoing paragraphs, and in no case could fat be demonstrated in the alveolar spaces. With death caused by overventilation, immersion followed by dyspnea, or asphyxia accompanied by hyperpnea and dyspnea or without dyspnea, stains for fat always gave negative results. After the insufflation of various substances, fat-free in themselves, similar negative results prevailed.

*Evidence That Fat Is from Exudate.*—The only other explanation of the presence of fat in the membranes in certain of the cases of streptococcic and influenzal pneumonia must depend on the production of fat from the purulent exudate in the lungs. There is abundant evidence for this supposition. In 1,000 parts of dried pus cells, there are 75 parts of fat and like amounts of lecithin and cholesterol. In addition, pus-containing serum shows approximately 1.2 per cent fat, cholesterol and lecithin, with fat representing 0.5 per cent (Wells<sup>4</sup>). Small amounts of fat are present in any pneumonic exudate (MacCallum<sup>5</sup>).

#### ARTIFICIAL PRODUCTION OF HYALINE MEMBRANE

The final problem was the artificial reproduction of an eosin-staining hyaline membrane. From histologic appearances and staining reactions it had been concluded that the eosin stain was due to the nature of the exudate and that of the débris included in the membrane and to the degree of autolysis which the exudate had undergone.

Fibrinopurulent exudate from a case of empyema was mixed with physiologic solution of sodium chloride and injected into the tracheas of one live and one dead rabbit, while the animals were subjected to forceful artificial respiration in the Drinker apparatus. Microscopically, the exudate was found in the alveolar spaces of both animals, often in membrane formation against alveolar walls. The membranes consisted of leukocytes and fibrin, and only in occasional small areas were they typically eosin-stained. Similar exudate was then allowed to stand in an incubator at 40 C. for two days, following which the material was injected as before into the tracheas of both *dead* and *live* rabbits, which were subjected to artificial respiration. Microscopically, the material was found scattered throughout the air spaces in all lobes of both lungs. Material was gathered, in part, in small patchy areas in the alveolar spaces and ducts. More often, however, the injected material was found pressed against alveolar walls in amorphous masses in which no cellular detail could be recognized. This material stained definitely

4. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

5. MacCallum, W. G.: Textbook of Pathology, ed 4, Philadelphia, W. B. Saunders Company, 1928.



pink to red in the hematoxylin and eosin stains, and by the phosphotungstic acid hematoxylin stain was negative for fibrin. The picture could not be distinguished from the hyaline membrane found so characteristically in influenzal pneumonia (figs. 4 and 5).

#### SUMMARY

Membrane-like structures lining alveolar walls and identical in position and shape but not in staining reaction with the so-called hyaline membrane of influenza were found, on histologic examination, in the lungs of patients dying from a variety of causes. The presence of

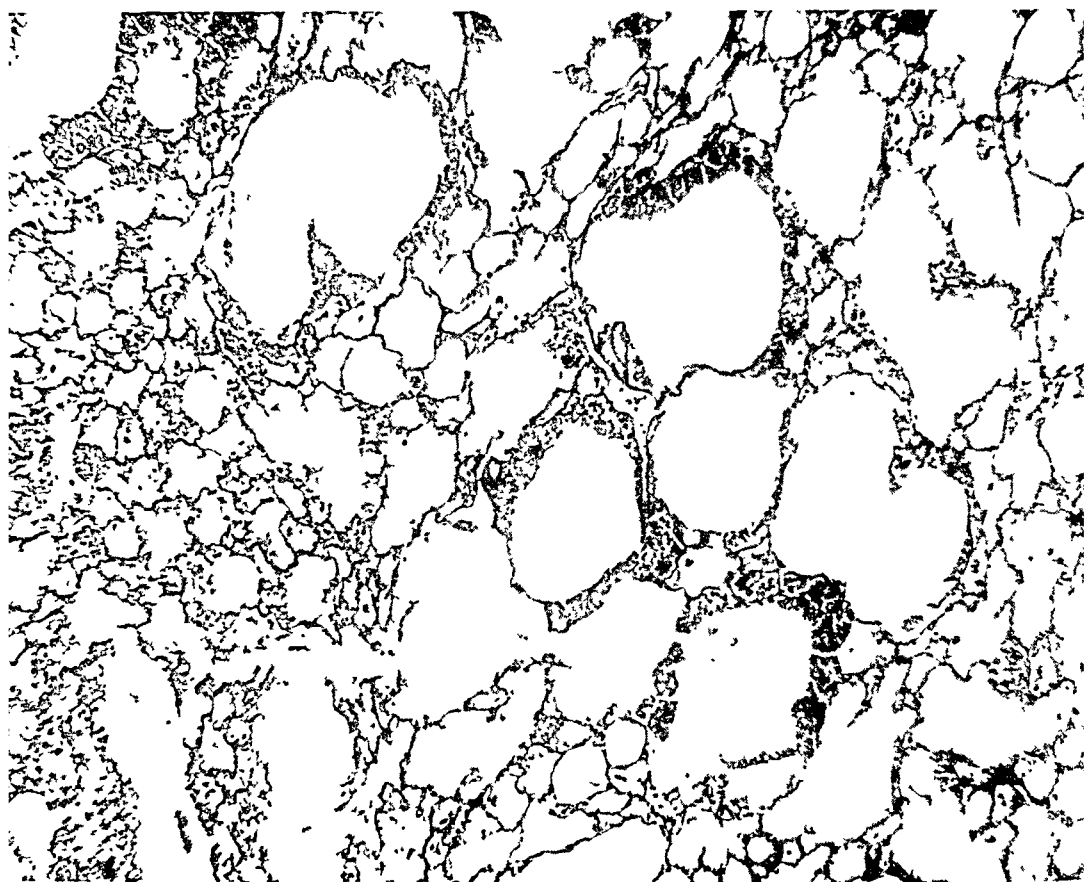


Fig. 4.—Photomicrograph of lung from a rabbit. Autolysed fibrinopurulent exudate was injected into the trachea after the animal had been killed. Artificial respiration was then carried out in the Drinker respirator. Note the position of the injected material in typical membrane formation. Aniline blue; reduced from a magnification of  $\times 112$ .

fluid matter in the air passages, causing partial obstruction to the passage of air, and marked dyspnea characterized the deaths of all these patients.

The fat in the membranes in certain of the cases of influenzal and streptococcic pneumonia is best explained as fat liberated from the exudate as a result of autolysis. It was found impossible to draw fat

into the alveolar spaces from the blood stream by reproducing in a number of ways the important mechanical factors that are present in every case in which the membrane is found.

When animals were forced to breath an atmosphere of such low oxygen and high carbon dioxide that marked dyspnea leading to death occurred, the alveolar spaces were filled with varying amounts of serum frequently pressed by the inspired air into membrane formation against the alveolar walls. When the carbon dioxide was removed so that little

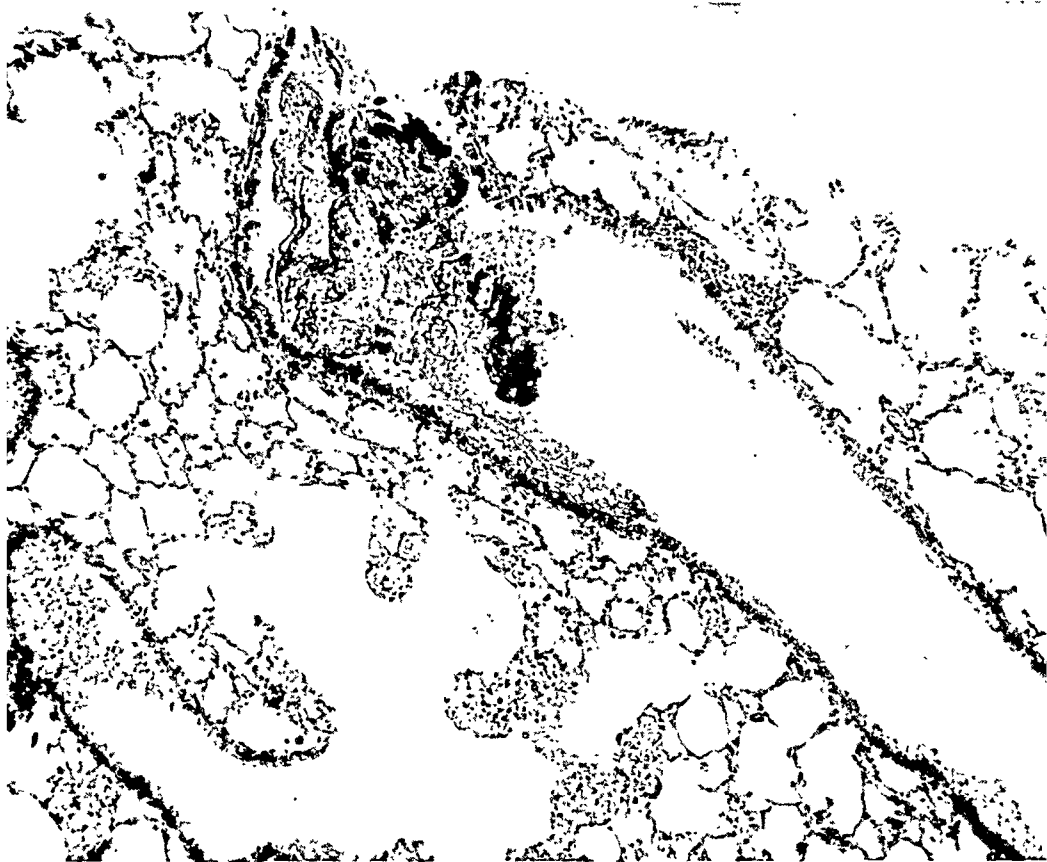


Fig. 5.—Photomicrograph of lung and bronchus from the rabbit described in the legend for figure 4. Note the hyaline, eosin-staining membrane near the wall of the bronchus. Hematoxylin and eosin; reduced from a magnification of  $\times 125$ .

dyspnea occurred before death from anoxemia, serum was found in the alveoli but no membrane formation.

When a foreign substance, such as horse serum, india ink or fibrino-purulent exudate, was instilled into the trachea and vigorous artificial respiration (in a Drinker respirator) instituted, characteristic membranes formed of the material could be found lining the alveolar walls.

That the living animal is not necessary for the production of the membrane, and that only the correct mechanical conditions are of

importance, was shown by the production of membranes in the lungs of dead animals subjected to artificial respiration during the intra-tracheal injection of various foreign materials.

The absence of membranes in a mechanically produced mediastinal emphysema where no exudate was present, and the production of such membranes in artificial emphysema caused by the injection of both air and exudate into the subcutaneous tissues of an animal, stress the necessity of having both factors present for the formation of a membrane. These experiments recall the finding by Wolbach and by ourselves<sup>1</sup> of typical membranes in mediastinitis with emphysema.

The eosin-staining character of the typical membrane, as seen in influenzal and streptococcic pneumonia, was found to depend on the degree of autolysis of the exudate incorporated therein. It was possible by injecting suitable exudate into the tracheas of *dead* animals subjected to artificial respiration to produce typical eosin-staining membranes lining the walls of bronchioles and alveolar spaces.

# THE BRAIN STEM IN PNEUMONIA

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There has been for many years a feeling that death in acute infections, particularly in pneumonia, is determined primarily by a failure of control of respiration and circulation by the central nervous system.<sup>1</sup> There is considerable physiologic evidence to support this theory.<sup>2</sup> No histologic studies of the effect of pneumonia on the nerve centers recognized as governing these functions have been found in the literature, although a few such studies are available on the effect of experimental anoxemia, electrocution and death by freezing.<sup>3</sup> The principal centers supposed to regulate these functions through both the somatic and the autonomic system are:

1. The motor nucleus of the trifacial nerve, the nucleus of the facial nerve, the hypoglossal nucleus, the nucleus ambiguus, the spinal accessory and anterior horn cells supplying somatic motor fibers, from large polyhedral cells well supplied with Nissl substance.

2. The vagus motor nucleus and sacral autonomic cells supplying principally autonomic motor fibers to smooth muscle, from cells of moderate size with somewhat less Nissl material.

3. The indefinitely located respiratory and vasomotor centers of association, lying supposedly in the pons and the medulla oblongata.

4. The central connections of the sympathetic system, thought to lie in the diencephalon (Bard<sup>4</sup>). They are not considered here, because these areas have not been studied. However, one recently studied brain, in which sections of the hypothalamus were made, showed areas of nerve cell degeneration in this region similar to that to be described for the more damaged portions of the brain stem.

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1. Weiss, Soma: *Ann. Int. Med.* **5**:100, 1931. Janeway, T. C.: *New York State J. Med.* **85**:193, 1907. Crile, G. W.: *J. A. M. A.* **76**:149, 1921. Mott, Fred: *Lancet* **1**:519, 1921.

2. Keller, A. D.: *Am. J. Physiol.* **96**:59, 1931. Newburgh, L. H.; Means, J. H., and Porter, W. T.: *J. Exper. Med.* **24**:583, 1916. Porter, W. T.; Newburgh, L. H., and Newburgh, I.: *Am. J. Physiol.* **35**:1, 1914. Boothby, W. M., and Shamoff, V. N.: *ibid.* **37**:418, 1915-1916. Atchley, D. W.: *J. A. M. A.* **95**:385, 1930. Kastlin, G., and MacLachlan, W. W. G.: *Ann. Int. Med.* **4**:959, 1931.

3. Chornyak, John, and Sayers, R. R.: *Pub. Health Rep.* **46**:1523, 1931. Morrison, L. R.; Weeks, A., and Cobb, S.: *J. Indust. Hyg.* **12**:324 and 364, 1930. Urquhart, R. W. I.: *ibid.* **9**:140, 1927. Bender, L.: *Arch. Neurol. & Psychiat.* **20**:319, 1928.

4. Bard, P.: *Arch. Neurol. & Psychiat.* **22**:230, 1929.

The vasomotor center is supposed to lie somewhere in the medulla oblongata beneath the floor of the fourth ventricle. The respiratory center, according to Cajal (Ranson<sup>5</sup>), lies in the posterior commissure of the nucleus of the tractus solitarius, but opinions of Legalois and Flourens, Schiff and Girard, Mislavski, Gad and Marinesco, and Bectereu (Tilney and Riley<sup>6</sup>) place it in the reticular gray matter of the pons and medulla. Lumsden,<sup>7</sup> in his physiologic experiments, divided the respiratory center into four parts, a regulatory center in the upper part of the pons, a center of inspiratory tone and an expiratory center in the upper part of the medulla near the level of the striae acousticae and a gasping center at the posterior limit of the fourth ventricle. The work of Lumsden will be discussed in detail in connection with the present work.

Function and failure of the respiratory mechanism are so closely linked with function and failure of the vasomotor mechanism that it seems likely, although unproved, that the anatomic centers of the control of these mechanisms lie in great proximity to each other. No attempt will be made to separate them, although it would appear that of the two, the more fundamental to life is the respiratory, since the vasomotor mechanism is developed secondarily, to meet the primary need for internal respiration in the multicellular organism.

#### MATERIALS AND METHODS

The present study deals with observations on the medullary and pontile centers in five cases of lobar pneumonia (one of them complicated by endocarditis and meningitis) and one of bronchopneumonia in man and one case of experimental bronchopneumonia (pneumococcic) in the rabbit. The control material consisted of two human brains (fixed two hours post mortem) from subjects of legal electrocution, six rabbit brains (fixed at various periods post mortem) and twenty-two mouse brains. The latter came from mice that had died of pneumococcic peritonitis or from normal mice, and were fixed either immediately or several hours post mortem. All tissue was fixed with Carnot's solution, and stains were made in duplicate with toluidine blue and with toluidine blue and phloxin. The animals were killed by a large intracardiac injection of a saturated solution of magnesium sulphate. The pons and the medulla oblongata in each case were fixed in successive disks averaging 3 or 4 mm. in thickness. The time of fixation in the

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5. Ranson, S. W.: *The Anatomy of the Nervous System*, ed. 2, Philadelphia, W. B. Saunders Company, 1925, p. 330.

6. Tilney, F., and Riley, H. A.: *The Form and Functions of the Central Nervous System*, New York, Paul B. Hoeber, Inc., 1921, p. 324.

7. Lumsden, Thomas: *J. Physiol.* **57**:153 and 354, 1923; **58**:81 and 111, 1923.

human cases ranged from one to three hours post mortem. All sections were made from as near the surface of the blocks as it was possible to get complete sections.

#### THE BRAIN STEM OF THE MOUSE

The first studies were made on the brain stems of mice. Because of the technical difficulties, the small size of the nerve centers and the difficulty in handling, observations on the twenty-two mice yielded only two results of value: (1) instruction in the technic of preparation to insure uniformity of fixation and stain, and (2) the knowledge that up to two or three hours after death postmortem changes in the nerve cells, while in the later hours recognizable, are very slight and very different from the damage to be described in the pneumonic patients.

#### THE BRAIN STEM OF THE RABBIT

The medullary and pontile centers of six rabbits were studied. The observations were as follows:

*Changes in the Cells.*—1. Normal Rabbit (1); Immediate Fixation: The cells of the various nuclei were in extremely good preservation (fig. 1 A).

2. Normal Rabbits (2 and 3); Fixation Two and Four Hours Post Mortem: At two hours, there was little change; this consisted of slight perineural retraction in places and slight fuzzing and finer granularity of Nissl bodies, but within normal limits, so that, without labeling, sections could not be distinguished from those from an animal on which autopsy had been performed immediately (fig. 1 B). At four hours, the changes were similar to those at two hours, but more marked, so that cells could be definitely recognized as abnormal. Most cells, however, even at four hours showed remarkably little change, in spite of obvious changes in the white matter (fig. 1 C).

3. Rabbits (4, 5 and 6) Exposed to Carbon Dioxide (25 per cent) and Oxygen (75 per cent) for Two Hours; Immediate Autopsy: Very slight but recognizable changes were present in the Nissl substance, especially in the cells of the gray reticular substance, consisting of slight hyperchromatism or of chromatolysis in the early stages, similar to that seen post mortem in the normal rabbits. At two and four hours post mortem, these changes were slightly more marked but of the same character as the postmortem changes in the normal rabbits. (Carbon dioxide was given with 75 per cent oxygen, an amount far in excess of that necessary to prevent anoxemia. Occasional areas of chronic encephalitis were encountered in several of the rabbits, but the regions of these areas were strictly avoided in making observations.)

*Other Changes.*—These consisted of moderate vascular engorgement in the animals given carbon dioxide and varying amounts of vacuolization (false por-encephalia) and axon swelling in the white matter due to postmortem disintegration.

Important Indications from These Data.—1. Postmortem changes in the cells of the brain stem of the rabbit up to two or three hours are so slight as to be almost negligible with the present method of prepara-

tion, and what changes there are, are recognizable. 2. In the rabbits exposed to carbon dioxide in an effort to exhaust respiratory neurons, there is a hint of particular damage to the *large and small cells of the reticular gray matter*. (See discussion of human material with particular reference to that of the respiratory center.)

#### THE BRAIN STEM OF MAN

Material from the human brain stem was studied in the following cases:

1. Woman, aged 65; lobar pneumonia; fixation one and one-half hours post mortem
2. Man, aged 35; lobar pneumonia; fixation one and one-half hours post mortem

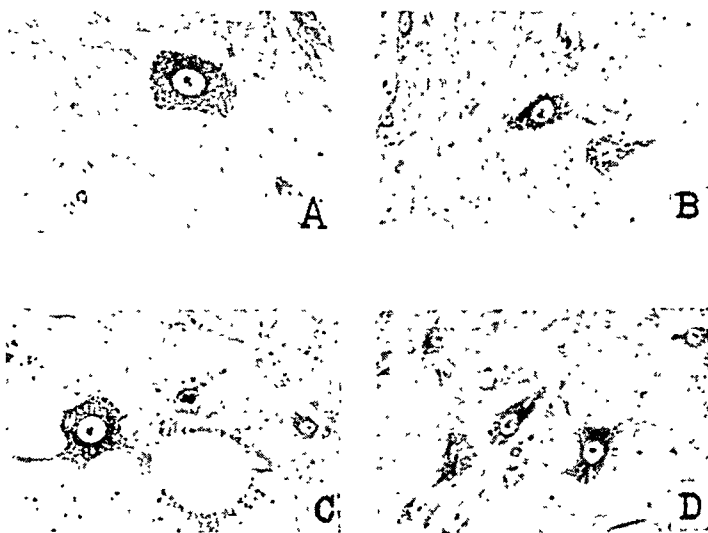


Fig 1.—Cells from the reticular gray matter of the medulla of the rabbit brain: *A*, photomicrograph of a normal large cell (normal rabbit), fixed immediately after the rabbit was killed; *B*, appearance of smaller cells, normal except for slight changes in the Nissl substance—from a normal rabbit, the tissues of which were fixed two hours post mortem; *C*, a large cell with slight change in the Nissl substance and slight perineural retraction, but with the structure remarkably well preserved—from a normal rabbit, the tissues of which were fixed four hours post mortem; the white matter shows large vacuoles; *D*, smaller cells showing loss of definition of the Nissl granules and slight nuclear changes—from a rabbit dead of bronchopneumonia, the tissues of which were fixed immediately.

3. Woman, aged 36; lobar pneumonia; fixation three hours post mortem
4. Woman, aged 31; bronchopneumonia; fixation three hours post mortem
5. Man, aged 57; lobar pneumonia; fixation two hours post mortem
6. Man, aged 57; lobar pneumonia, pneumococcic endocarditis (tricuspid) and pneumococcic meningitis; fixation one hour post mortem
7. Rabbit, adult; bronchopneumonia; immediate autopsy and fixation

The control tissue was from electrocuted persons and was fixed two hours post mortem.

The changes to be described are only those uniformly present throughout all six cases and are not of the type due to postmortem disintegration, seen in rabbits. The pneumonic rabbit showed slight changes consistent with those found in the human cases (fig. 1 *D*).

*Changes in Cells (Exclusive of Pigmentation).*—1. Sensory Nuclei (Nucleus Cuneatus, Nucleus Gracilis, Sensory Root of Fifth Nerve, Vestibular and Cochlear Nuclei): The control showed moderate shrinkage of cells and perineural retraction (edema?) with some decrease in Nissl substance and occasional cells with chromatolysis. The changes have the appearance of the postmortem changes seen in rabbits. The pneumonic person showed marked shrinkage of cells, perineural retraction, and chromatolysis that left shapeless, hyperchromatic masses as remnants of many of the cells. These changes were much more pronounced than in the control.

2. Somatic Motor Nuclei (Anterior Horn and Spinal Accessory Cells, Nucleus Ambiguus, Hypoglossal Nucleus, Nucleus of Facial Nerve, Nucleus Abducens):

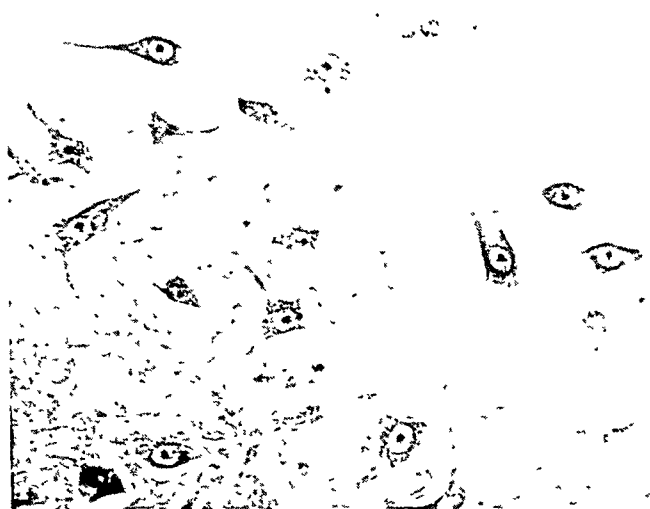


Fig. 2.—Somatic motor cells (hypoglossal nucleus) of the human brain stem from a person who died of lobar pneumonia. The cells are relatively intact, except for perineural retraction and occasional cell disintegration.

The control showed excellent preservation of cells except for slight shrinkage and perineural retraction. The Nissl substance was almost perfectly preserved. The pneumonic person showed the cells in good preservation, almost as good as in the controls, but with more perineural retraction (fig. 2).

3. Visceral Motor Nuclei (Vagus Motor Nucleus, Nucleus of Tractus Solitarius): The control revealed moderate loss of Nissl substance and an occasional shrunken cell. The cells were generally well preserved. The changes were probably due to postmortem disintegration. The pneumonic person's cells were similar to those of the control with slightly more change of the same character. The lesion was interpreted as an early postmortem change plus some general toxic damage.

4. Cells of Reticular Gray Matter (To Be Discussed): The control presented slight shrinkage of these cells and perineural retraction. The Nissl substance was fairly well preserved (figs. 3 and 4 *A*). The pneumonic person showed marked



swelling of large and small cells with rounding of the cell outlines, chromatolysis and migration of the nuclei to the cell margins, with shrinkage and condensation of the nuclei. The changes were most marked in the upper medullary (middle) expansion, but were present in corresponding levels of expansion in the midmedulla (lower expansion) and upper part of the pons (upper expansion) (figs. 3 and 4 *B*).

5. Cells of Central Gray Matter of Upper Part of Pons (Floor of Fourth Ventricle and Floor of Aqueduct of Sylvius Central to Locus Caeruleus, and Nucleus of Median Raphe of Upper Part of Pons) (To Be Discussed with Section 4): The controls showed little change except for pigment (see later paragraphs on this), which was present to some extent. The pneumonic person showed swelling and irregularity of cell outlines, with migration of the nuclei to the cell margins and chromatolysis.

6. Olivary Bodies and Others: The controls showed changes similar to those in the sensory cells, but more marked. The pneumonic person also showed changes similar to those in the sensory cells, but more marked.

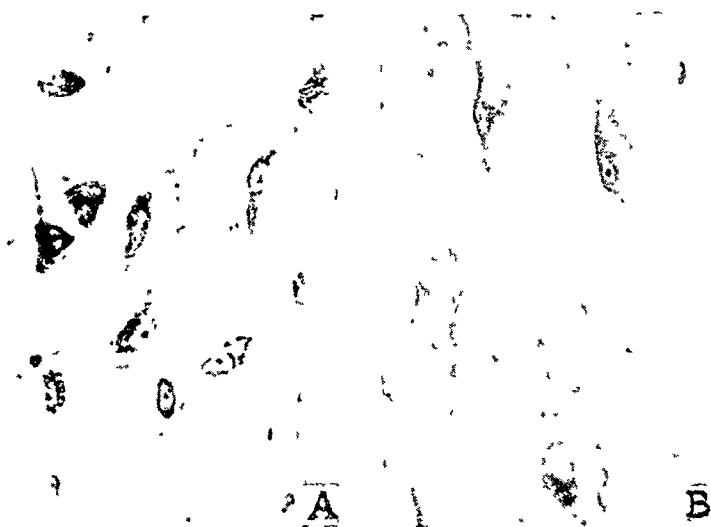


Fig. 3.—Cells of the reticular gray matter at the level of the upper part of the medulla oblongata in the human brain stem: *A*, relatively well preserved cells from a control; *B*, a corresponding area from the brain stem of a person who died of lobar pneumonia, showing swelling of cells, chromatolysis, nuclear displacement but with shrinkage of the nuclei and little perineural retraction.

Important Indications from These Data.—1. An extremely slight, though general, lessening of the sharp definition of the Nissl granules was present throughout all sections and was interpreted as representing the general toxic damage seen in all other tissues of the body (so-called cloudy swelling).

2. Varying amounts of perineural retraction (edema?) were seen, most marked in the sensory centers and least marked in the somatic motor cells. The assumption that the enlarged perineural spaces were the result of edema was borne out, as in Chornyak and Sayer's<sup>3</sup> work, by the presence of perivascular edema as well. The same appearance

was noted by Kubie<sup>8</sup> in his hydration experiments on animals. It must be remembered, however, that if the tissue is not perfectly fixed, cell shrinkage may occur which may simulate edema closely. The same observation was occasionally made in animals examined long post mortem, but not with any regularity in those examined within the periods of time of the human cases. The cells of the vagus motor nucleus, the nucleus ambiguus and the nucleus of the tractus solitarius showed slightly more damage than did the motor cells, but less than the sensory cells.

3. A *rather vaguely defined nucleus* in the reticular gray matter, made up of a column of cells running from the peduncles downward and outward to a position in midmedulla between the nucleus ambiguus and

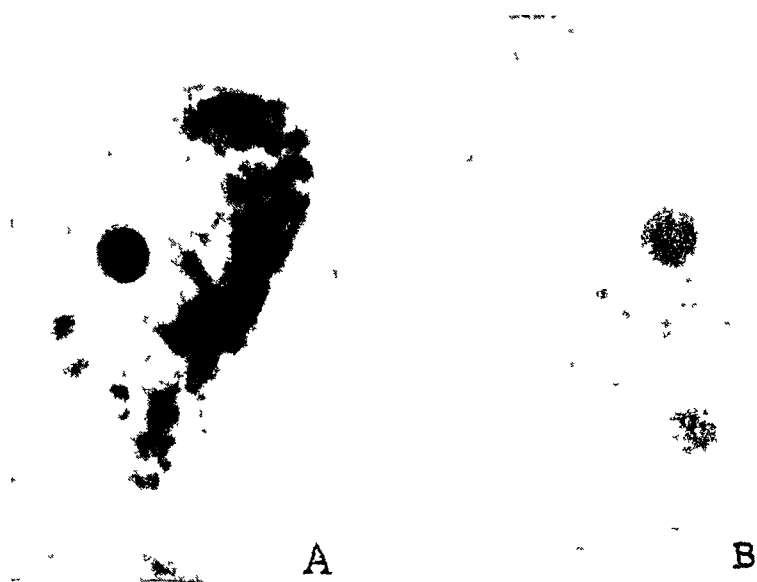


Fig. 4.—Cells from fields *A* and *B* of figure 3, seen by oil immersion. Note the relative integrity of the Nissl granules, nucleus and cell outline in the control (*A*) as compared with the marked destruction in the pathologic tissue (*B*). Observe the collection of golden-brown pigment at one pole of the damaged cell.

the dorsal accessory olive and beyond, was noted particularly. This column appeared to have enlargements (*a*) at the upper level of the pons, (*b*) at a level immediately caudal to the striae acousticae and (*c*) at the lower limit of the inferior olive, at the pyramidal decussation just internal to the spinal accessory motor cells. The cells of this column showed extreme degenerative changes, comprising marked swelling, partial or complete chromatolysis and eccentric, distorted nuclei. There was little perineural retraction here. The changes in this group of cells will be discussed in detail in later paragraphs. Extension of this group

8. Kubie, L. S.: Brain 51:244, 1928

of cells into the floor of the aqueduct of Sylvius, medial to the cells of the locus caeruleus, and the nucleus of the median raphe also showed a marked degree of the same type of damage.

*Pigmentary Changes.*—1. Normal Pigment: This was seen in cells of the locus caeruleus (substantia ferruginea) and in occasional scattered cells throughout. It appeared as coarse brownish-black granules loading the cytoplasm of the cells. It was seen in both animal and human material, and in both control and pathologic tissue.

2. Abnormal Pigment: Delicate golden-brown pigment occurred in masses of extremely fine granularity in the cytoplasm of many of the neurons, displacing and often replacing the Nissl substance. It was abundant in badly damaged cells and in cells of older persons and was most marked in those of a partially demented person who died of pneumonia.

This golden pigment was much more abundant in cells of persons who died of pneumonia than it was in the cells of controls of the same range of age. However, the amount of chromatolysis and nuclear damage was not always proportionate to the amount of pigment. The pale-golden pigment has been said by various European authors (for instance, Kaufmann<sup>9</sup>) to be present more commonly in persons of advanced age, in demented persons and in those dying of acute infectious diseases, particularly typhoid fever. It is supposed to represent an accumulation of uneliminated waste products. Its exact character is not known, although probably it belongs to the lipochromes. It was never seen in animal tissue.

*Other Changes.*—1. Moderate engorgement of blood vessels was noted, with edema of perivascular spaces.

2. Occasional vacuolization of white matter was seen in both control and pathologic tissues, but it seemed to have no relation to cell preservation.

3. One pneumonic person and one control showed slight perivascular round cell infiltration in the meninges (probably syphilis or, in the pneumonic person, a feature of the "toxic encephalosis" described by Winkelman,<sup>10</sup> without apparent influence on the nerve cell changes.

4. One pneumonic person showed a massive polymorphonuclear meningeal exudate, *but the cells of the parenchyma showed only the same changes as did those of pneumonic persons without meningitis.*

5. Practically no increase in satellite glia cells was found.

#### COMMENT

The first point essential in dealing with human tissue is the recognition of the normal conditions and of the character and time of appearance of postmortem changes, since human material can practically never

9. Kaufmann, Edward: Pathology for Students and Practitioners, translated by Stanley P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 3, p. 1871.

10. Winkelman, N. W.: Pennsylvania M. J. **33**:208, 1930.

be fixed as rapidly after death as that from the experimental animal. The work on the mice and rabbits made me realize two points: (1) that in brain tissue up to two to three hours after death changes due to postmortem disintegration were slight and were recognizable, and (2) that the profound changes seen in the cells of the human material were emphatically not due to postmortem disintegration. It must be remembered that human subjects of electrocution are supposed to die as the result of central respiratory failure, so that the control tissue might be expected to show some changes in the nuclei associated with respiratory control. The rapidity of death probably accounts for the fact that these cells were so little damaged.

Secondly, it was very striking that these changes, with certain exceptions to be noted, were identical, though less intense, with the results of Chornyak and Sayer<sup>3</sup> in animals asphyxiated by carbon monoxide. The changes were not so profound as those that they observed, but the anoxemia of pneumonia is much less intense than that to which their animals were subjected. It is further interesting that in the rabbits subjected to carbon dioxide, with plenty of oxygen to prevent cellular asphyxia, these changes did not occur except for slight damage to the group of cells of the reticular gray matter. The one outstanding observation in the human material differing from Chornyak and Sayer's report was that only slight change was seen in the cells of the vagus motor nucleus and the nucleus of the tractus solitarius as opposed to the profound disturbances observed by them. This may perhaps be explained by the less intense degree of a similar damaging agent (namely, anoxemia) acting on cells perhaps a little more resistant in man than in the dog. It is possible, too, that certain cells are essentially more susceptible to damage of *any* sort than others, and that much the same changes are found regardless of whether the damaging agent is anoxemia or bacterial toxemia. Certainly it appears that in pneumonia the most profound nerve cell damage occurs in almost the same areas as those so severely damaged by simple anoxemia.

Particular attention must now be called to that irregular column of cells in the gray reticular substance. Chornyak referred to the central expansion of this "nucleus" as an unidentified nucleus lying in the gray reticular substance between the dorsolateral accessory plate of the inferior olive and the nucleus ambiguus, and described profound damage to these cells as compared with closely adjacent ones. In the human series I observed very severe damage to these cells and to corresponding ones in the upper and lower expansions of this mass as well. When one remembers that the consensus places the respiratory center just in this area, it becomes increasingly interesting. Moreover, Lumsden<sup>7</sup> demonstrated in the mammal that physiologically the respiratory mechanism can be divided into four philogenetic levels: (a) a regulatory,

center in the upper part of the pons (upper expansion?), (b) an inspiratory and expiratory center close together at the level of the striae acousticae and just below it (middle expansion?) and (c) a gasping center near or caudal to the posterior limit of the fourth ventricle (posterior expansion?). A combined view of these bits of evidence gives one a strong suggestion that this tripartite "nucleus" may be the actual center of the respiratory and perhaps of the vasomotor mechanism, and that it bears *extremely severe damage* both in pure anoxemia and in pneumonia.

In comment I do not wish to intimate that the brain is the only organ damaged by the noxious agents in either pneumonia or anoxemia. I believe that the liver, peripheral blood vessels and kidneys are greatly damaged, together with all the tissues of the body, and that malfunction of these probably adds the toxic products of faulty function and metabolism to those of bacterial origin.

#### CONCLUSIONS

In death from pneumonia (six cases) there was marked damage to the cells of the medullary and pontile centers, the cells of the various nuclei showing varying degrees of change. The most severe and the least severe damage occurred in corresponding nuclei in the six cases. Cells in the sensory nuclei showed more damage than those in the motor nuclei. Cells of the reticular gray matter, which has been described by others as the probable site of the respiratory and vasomotor centers, showed the most severe degenerative changes.

# VIOSTEROL IN EXPERIMENTAL FIBROUS OSTEITIS

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Recent study of osteitis fibrosa cystica has shown this condition to be a manifestation of hyperfunction of the parathyroid glands (Barr and Bulger;<sup>1</sup> Hunter and Turnbull<sup>2</sup>). Experimental proof of this relationship was first offered by Jaffe, Bodansky and Blair,<sup>3</sup> who produced the lesions of fibrous osteitis in young guinea-pigs and dogs by injections of parathormone. We began the investigations reported in this paper with the objects of reproducing the work of Jaffe and his co-workers and of discovering whether the administration of viosterol would prevent the bone lesions induced by parathormone.

## METHODS

Young guinea-pigs weighing between 200 and 350 Gm. were used. The diet consisted of oats, hay, carrots, cabbage and water, ad libitum. The animals were weighed once a week. Parathormone was injected subcutaneously. Viosterol 250 D was diluted in maize oil so that the desired dosage was contained in 0.2 cc. It was given orally by means of a Luer syringe. The animals received viosterol for ten days before the injections of parathormone were begun. At the end of the experiment, they were bled to death and examined immediately. The bones were fixed in Zenker's solution plus 5 per cent glacial acetic acid, and decalcified in Müller's fluid. Paraffin sections of the ribs and long bones were stained with hematoxylin and eosin.

The soft tissues were fixed in alcohol, and paraffin sections were stained by the von Kossa method as well as in hematoxylin and eosin. However, no calcium deposits or other changes relevant to this work were observed.

The parathormone used in this work was donated by Eli Lilly & Company. Mead Johnson & Company supplied the viosterol.

## PRODUCTION OF THE LESIONS OF FIBROUS OSTEITIS

Animals 1, 2, 3, 4 and 5 were used in this experiment. The schedule of the daily doses of parathormone is given in the table. Animals 2 and 3 each received only 326 units of parathormone over a period of twenty-five days. The bone changes were comparatively slight. In the ribs, there was some thinning of the cortex with

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From Beth Israel Hospital.

1. Barr, D. P., and Bulger, H. A.: *Am. J. M. Sc.* **179**:449, 1930.

2. Hunter, D., and Turnbull, H.: *Brit. J. Surg.* **19**:203, 1931.

3. Jaffe, H. L.; Bodansky, A., and Blair, J. E. *Arch. Path.* **11**:207, 1931; *J. Biol. Chem.* **88**:629, 1930; *J. Exper. Med.* **52**:669, 1930.

## Dosage Schedules

Guinea-Pig	Units of Parathormone Administered on Given Day of Injection																															Daily Dose of Viosterol, Mgs.	Total Units of Parathormone	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31			
1	6	6	8	9	10	10	10	10	10	15	15	20	20	20	25	25	28	30	40	50	50	50	60	60	40	40	K	..	..	..	0	581		
2	4	4	4	4	4	4	4	8	10	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0	K	..	0	326	
3	4	4	4	4	4	4	4	8	10	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0	K	..	0	326	
4	4	6	6	10	10	10	10	10	15	20	25	30	30	40	0	40	0	0	0	20	10	10	10	10	20	20	20	20	K	..	..	0	466	
5	4	8	10	10	10	10	10	10	15	20	25	30	35	40	40	10	10	40	60	60	60	60	80	72	80	K	..	..	..	..	0	849		
6	4	6	6	8	10	10	10	10	15	20	25	30	30	10	0	40	0	0	0	20	10	40	10	20	20	20	20	K	..	..	20	466		
7	4	6	6	4	4	4	4	8	10	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0	K	20	345	
8	4	6	6	8	10	10	10	10	15	20	25	30	30	40	0	40	0	0	0	0	40	10	40	20	20	20	20	K	..	..	2	466		
9	4	6	6	4	4	4	4	8	10	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0	K	2	345	
10	4	6	6	10	10	10	10	10	15	20	25	30	30	40	0	40	0	0	0	20	40	10	40	20	20	20	20	20	20	20	0	K	8	466
11	4	4	4	4	4	4	4	8	10	10	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0	K	8	326
12	4	8	10	10	10	10	10	10	15	20	25	30	35	40	40	40	40	40	10	60	60	60	80	72	80	K	..	..	..	..	15	849		
13	4	6	6	8	9	10	10	10	10	15	15	20	20	20	25	25	28	30	40	50	50	50	60	10	10	K	..	..	..	..	15	581		
14	4	6	6	8	9	10	10	10	10	15	15	20	20	20	25	25	28	30	40	50	50	50	60	10	10	K	..	..	..	..	15	581		
15	4	6	6	10	10	10	10	10	15	20	25	30	30	40	0	40	0	0	0	20	40	40	10	20	20	K	..	..	..	..	15	466		
16	..	6	6	8	9	10	10	10	15	15	20	20	20	20	25	25	28	30	40	50	50	50	60	10	10	0	K	..	..	..	0	581		
17	4	6	6	10	10	10	10	10	15	20	25	30	30	40	0	10	0	0	0	20	10	10	10	20	20	20	K	..	..	..	0	161		
18	4	6	6	10	10	10	10	10	15	20	25	30	30	10	0	10	0	0	0	20	10	10	40	20	20	20	K	..	..	..	15	461		
19	10	0	10	20	0	0	10	0	0	60	0	0	80	0	0	80	0	0	0	80	0	0	K	..	..	..	..	..	..	..	0	380		
20	10	0	10	20	0	0	10	0	0	60	0	0	80	0	0	80	0	0	0	80	0	0	K	..	..	..	..	..	..	..	15	380		
21	10	10	10	20	30	40	0	50	0	60	0	80	0	10	20	10	10	10	10	10	10	10	10	10	10	10	K	..	..	..	0	480		
22	10	10	10	20	30	40	0	50	0	60	0	80	0	40	20	10	10	10	10	10	10	10	10	10	10	10	K	..	..	..	15	480		
23	10	10	10	20	30	40	0	50	0	60	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	K	..	..	..	0	310		
24	10	10	10	20	30	40	0	50	0	60	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	K	..	..	..	15	310		
25	10	10	10	20	30	40	0	50	0	60	0	80	0	K	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	310		
26	10	10	10	20	30	40	0	50	0	60	0	80	0	K	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	15	310		

evidences of subperiosteal resorption. The slightly widened haversian canals contained fibrous tissue. At the costochondral junction there was thinning of the trabeculae, which were surrounded by a few strands of fibrous tissue. The marrow showed some congestion. In the long bones, there was a relatively small amount of fibrous replacement of the marrow at the metaphysis close to the epiphyseal cartilage.

Animals 1 and 5 received 581 units and 849 units of parathormone, respectively, over a period of twenty-five days. The bones showed extremely severe lesions of fibrous osteitis. In the ribs, there was marked thinning of the cortex with fragmentation and deforming spontaneous fracture. The numerous osteoclasts in their deeply excavated Howship's lacunae further confirmed the active resorption of bone. Columns of closely packed fibrous tissue filled the widened haversian canals. At the costochondral junction, the trabeculae were fragmented and disorganized. Starting from this region and extending, especially in animal 5, far into the shaft, the marrow was almost completely obliterated by a large amount of dense connective tissue. The changes in the long bones were not as striking as those in the ribs. Although there were signs of subperiosteal resorption of bone, and although the widened haversian canals were filled with connective tissue, there was no obvious thinning of the cortex. The bone trabeculae, however, displayed definite decalcification. The marrow adjacent to the epiphyseal plate was usually replaced by fibrous tissue, which, however, did not extend into the shaft. No fibrosis of the marrow of the shaft proper was found.

Animal 4 received 466 units of parathormone over a period of twenty-six days. Lesions, equal in severity to those in animals 1 and 5, were found. In addition, at the costochondral junctions of the ribs, several definite cysts were observed embedded in dense fibrous tissue (fig. 1).

X-ray pictures of the animals in this group, taken after death, showed no changes in the bones.

The bone lesions produced by parathormone in the animals of this group agree in all essentials with those reported by Jaffe and his co-workers. However, although animals 4, 1 and 5 received different amounts of parathormone—466, 581 and 849 units, respectively—the lesions in all of them were of the same order of magnitude. It seems, therefore, that above a certain limit of dosage, the extent of the changes in the bones does not necessarily run parallel to the amount of parathormone injected.

#### EFFECT OF VIOSTEROL IN EXPERIMENTAL FIBROUS OSTEITIS

It is well known that adequate though relatively small doses of viosterol cause increased calcification of bone, at least in rickets and osteomalacia. Large doses of parathormone produce decalcification of bone. The possible antagonism between these two substances raised the question whether the administration of viosterol could prevent the decalcification and fibrous osteitis caused by parathormone.

The literature offers conflicting reports on this subject. Hoff and Homann<sup>4</sup> reported increased calcification, as judged by x-ray pictures,

4. Hoff, F., and Homann, E.: *Ztschr. f. d. ges. exper. Med.* **74**:258, 1930.



in the bones of adult rats from viosterol. Simultaneous injection of parathormone in another group of rats that received the same dose of viosterol prevented increased calcification of bone. No microscopic examinations or chemical analyses of the bones were made, and the x-ray picture, we hold, is not a delicate criterion of bone decalcification in



Fig. 1.—The costochondral junction of a rib of animal 4 under high power magnification. Note the cysts embedded in the fibrous tissue that has replaced the marrow.

small animals. The amount of viosterol, evidently 40,000 curative rachitic doses, given daily by these workers is highly toxic, and several of the animals died within a few days. That such massive doses of vitamin D should bring about calcification of bone is contrary to the findings of other workers, who almost uniformly report decalcification as

a result of such doses. Even though this objection is disregarded, the method used by Hoff and Homann is not a satisfactory one for demonstrating antagonism between parathormone and viosterol. It seemed better to attempt to counteract the decalcifying action of parathormone by means of viosterol than to prevent, by the use of parathormone, any possible increased calcification that might occur from vitamin D. Marked increase in calcification in normal animals from viosterol is certainly not a prominent finding. On the other hand, the decalcifying action of parathormone is definite and, within limits, may be brought to any desired degree.

The possible antagonism between these two substances has also been considered by clinicians. Regnier<sup>5</sup> observed a patient with von Recklinghausen's disease who was apparently benefited by vigantol. X-ray films demonstrated the improvement. Hunter and Turnbull<sup>2</sup> mentioned a possibly favorable effect in one patient following the use of ultraviolet irradiation and preparations of vitamin D. They suggested the use of viosterol when parathyroidectomy is contraindicated. Cystic degeneration of bone in the patient of Snapper and Boeve<sup>6</sup> progressed for seven weeks after parathyroidectomy, but was checked in two months by vigantol. Wilder<sup>7</sup> noticed improvement following the use of vitamin D in his case of generalized osteitis fibrosa cystica. Quick and Hunsberger<sup>8</sup> suggested a probable antagonism between vitamin D and the parathyroid hormone.

Other observations fail to indicate any therapeutic effect of vitamin D in osteitis fibrosa cystica. Snapper<sup>9</sup> found that viosterol did not influence the course of the disease. Prolonged use of large doses of cod liver oil, viosterol and ultraviolet irradiation failed in one of Hunter and Turnbull's<sup>2</sup> patients.

We attempted to discover whether the fibrous osteitis produced in guinea-pigs by parathormone could be prevented by viosterol.

Apparently rickets has never been produced in the guinea-pig, and viosterol has seldom been given to this animal. Kreitmair and Moll<sup>10</sup> used guinea-pigs in their studies of the toxic effects of massive doses of viosterol. We desired to avoid toxic dosages, as these bring about bone decalcification. Our dosages were arrived at as follows: Calculated on the basis of an average daily food consumption of 7 Gm., the minimum daily dose of viosterol, 250 D, that will cure rickets in rats is 0.17 mg. of the oily solution (McCollum's diet 3143 and five day test

5. Regnier, E.: *Fortschr. a. d. Geb. d. Röntgenstrahlen* **39**:696, 1929.

6. Snapper, I., and Boeve, H. J.: *Deutsches Arch. f. klin. Med.* **170**:371, 1931.

7. Wilder, R. M.: *Endocrinology* **13**:231, 1929.

8. Quick, A. J., and Hunsberger, A.: *J. A. M. A.* **96**:745, 1931.

9. Snapper, I.: *Arch. Int. Med.* **46**:506, 1929.

10. Kreitmair, H., and Moll, T.: *München. med. Wehnschr.* **75**:637, 1928.

period).<sup>11</sup> Since our guinea-pigs weighed approximately ten times more than the young rats used in the standardization of viosterol, we arrived at 1.7 mg. as a theoretically minimal basic dose. As it was desired to give sufficient vitamin D to produce an effect, doses varying from 2 to 20 mg. of viosterol were employed. These, it was felt from the work of Bills and Wirick<sup>12</sup> on rats, would entail no danger of toxic effects.

In this experiment, 9 animals were used (6, 7, 8, 9, 10, 11, 12, 13 and 14). All but animals 11, 10 and 6 received parathormone (table). These three animals were given viosterol alone in doses of 2, 8 and 20 mg., respectively. Microscopic examinations of the ribs and long bones revealed no abnormalities. Doses of parathormone similar to those given animals 2 and 3, i. e., from 326 to 345 units over a period of twenty-nine days, were given to animals 12, 14 and 8, which, in addition, received daily doses of 2, 8 and 20 mg. of viosterol, respectively. The mild lesions of bones observed in animals 2 and 3 were present here. Although the changes in the bones of 12, 14 and 8 may possibly have been less than in the two controls, the differences were too slight to be considered significant. Animals 7, 9 and 13, which received daily 2, 8 and 20 mg. of viosterol, respectively, were given the same schedule of parathormone dosage as guinea-pig 4, i. e., 466 units over a period of twenty-six days. In these three animals, lesions were almost completely absent in the ribs, and the changes in the long bones were much less than in the control. This apparent antagonism could not be demonstrated in later experiments, and we can offer no satisfactory explanation for its occurrence.

In order to obtain more definite and extensive information on the question of an antagonistic action between viosterol and parathormone another group of five animals was taken (15, 16, 17, 18 and 19). No. 19, serving as a control of the potency of the parathormone, received 581 units in twenty-five days, the same amount as guinea-pig 1 in a previous group. Typical and severe lesions of fibrous osteitis were produced. Animals 16 and 17 were similarly treated, but in addition received 15 mg. of viosterol daily. Striking lesions of fibrous osteitis were found and were of unusual severity, especially in the long bones. In fact, the changes in the long bones were the most pronounced we had yet encountered. The cortex was shattered and fragmented and, in certain areas, occupied by compact masses of fibrous tissue. Numerous osteoclasts bore evidence of active resorption of bone. The trabeculae had disappeared almost completely, and the fibrous replacement of the marrow had gone so far as to convey the impression that the entire metaphysis was one solid mass of connective tissue. Animals 18 and 15 received 15 mg. of viosterol daily in addition to 466 and 849 units of parathormone, respectively, over a period of twenty-five days. Severe lesions of fibrous osteitis were found, more extensive in the long bones than in the ribs. The changes in the long bones were, in fact, more pronounced in 18 and 15 than in controls 4 and 5, which received no viosterol.

In a group of ten animals, various modifications of the schedules of parathormone dosage were used in a further attempt to demonstrate a possible antagonism between this substance and viosterol. The animals were run in pairs, one receiving parathormone alone, and the other the same amount of parathormone plus 15 mg. of viosterol. Animals 20 and 21 were given 464 units of parathormone over a period of twenty-six days, the latter animal, in addition, receiving 15 mg. of viosterol daily. Severe lesions of fibrous osteitis were found in the ribs and long bones of both animals.

11. Bills, C. E.: Personal communication.

12. Bills, C. E., and Wirick, A.: *J. Biol. Chem.* 86:117, 1930.

It will be observed from the table that animals 22 and 23 received 380 units of parathormone in large doses at intervals over a period of twenty-two days. The animals were allowed to rest for from two to three days between the injections of 60 or 80 units of parathormone. No. 23 received, in addition, 15 mg. of viosterol daily. In animal 22, the lesions of fibrous osteitis were marked. In the ribs, the cortex was thinned, and the greatly widened haversian canals were filled with cel-

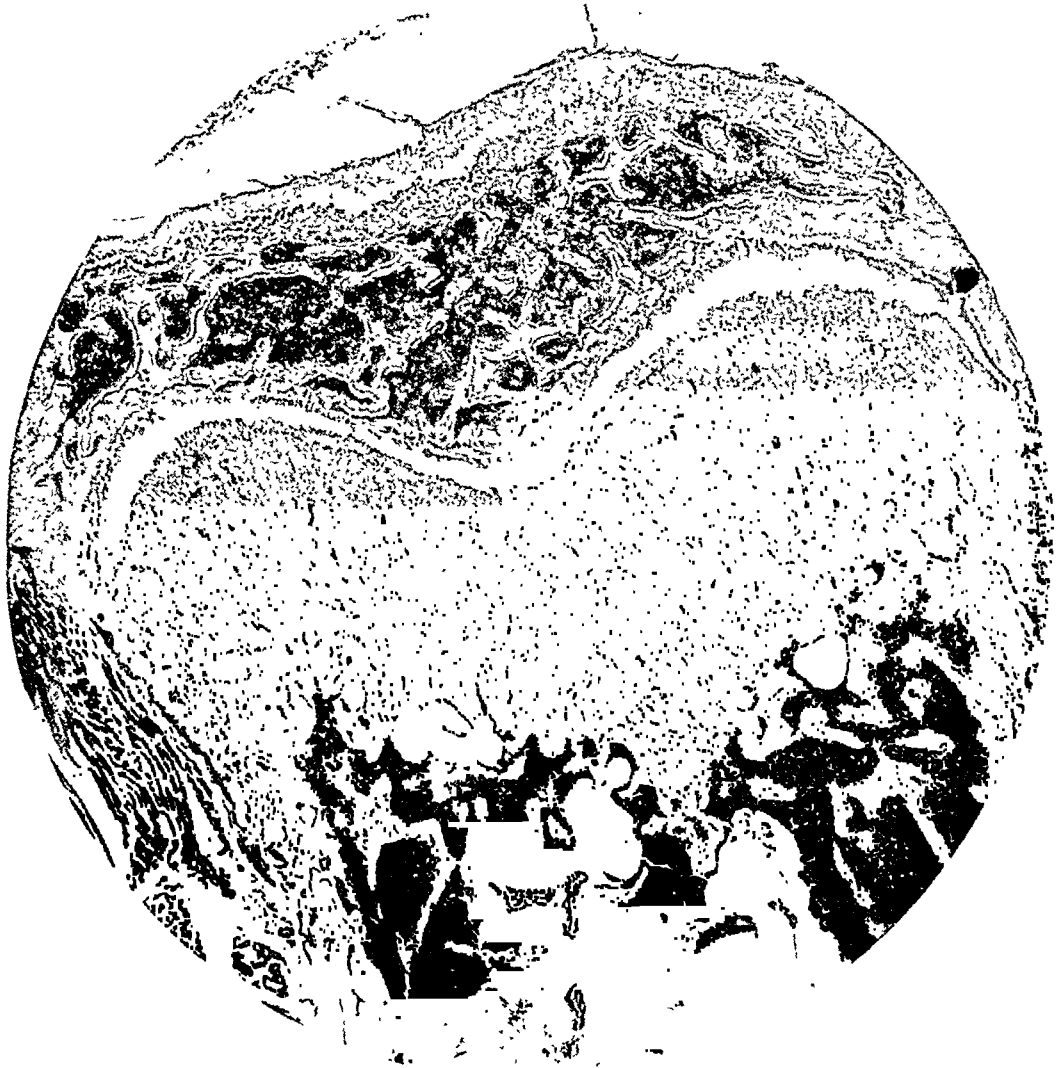


Fig. 2.—The proximal end of a tibia of animal 16. The bony trabeculae and marrow of the metaphysis are almost completely replaced by fibrous tissue.

lular connective tissue. Numerous osteoclasts were seen in the haversian canals as well as in the subperiosteal and endosteal regions. Similar evidences of resorption were visible around the trabeculae at the costochondral junction, and the marrow in this area was replaced by fibrous tissue. In the long bones, the greatly widened haversian canals were likewise packed with connective tissue. So marked was this process that in places the cortex was almost completely replaced by fibrous tissue. Numerous osteoclasts in Howship's lacunae surrounded the trabeculae and were present in the endosteal and subperiosteal regions. The usual replacement of the

metaphyseal marrow by fibrous tissue was observed. The marrow of the shaft was congested. A few islands of connective tissue could be discerned in the outer rim of the epiphysis. In animal 23, in spite of the daily administration of 15 mg. of viosterol, striking lesions of fibrous osteitis were present in the ribs and long bones. In fact, the resorption of bone and fibrous replacement of the marrow were

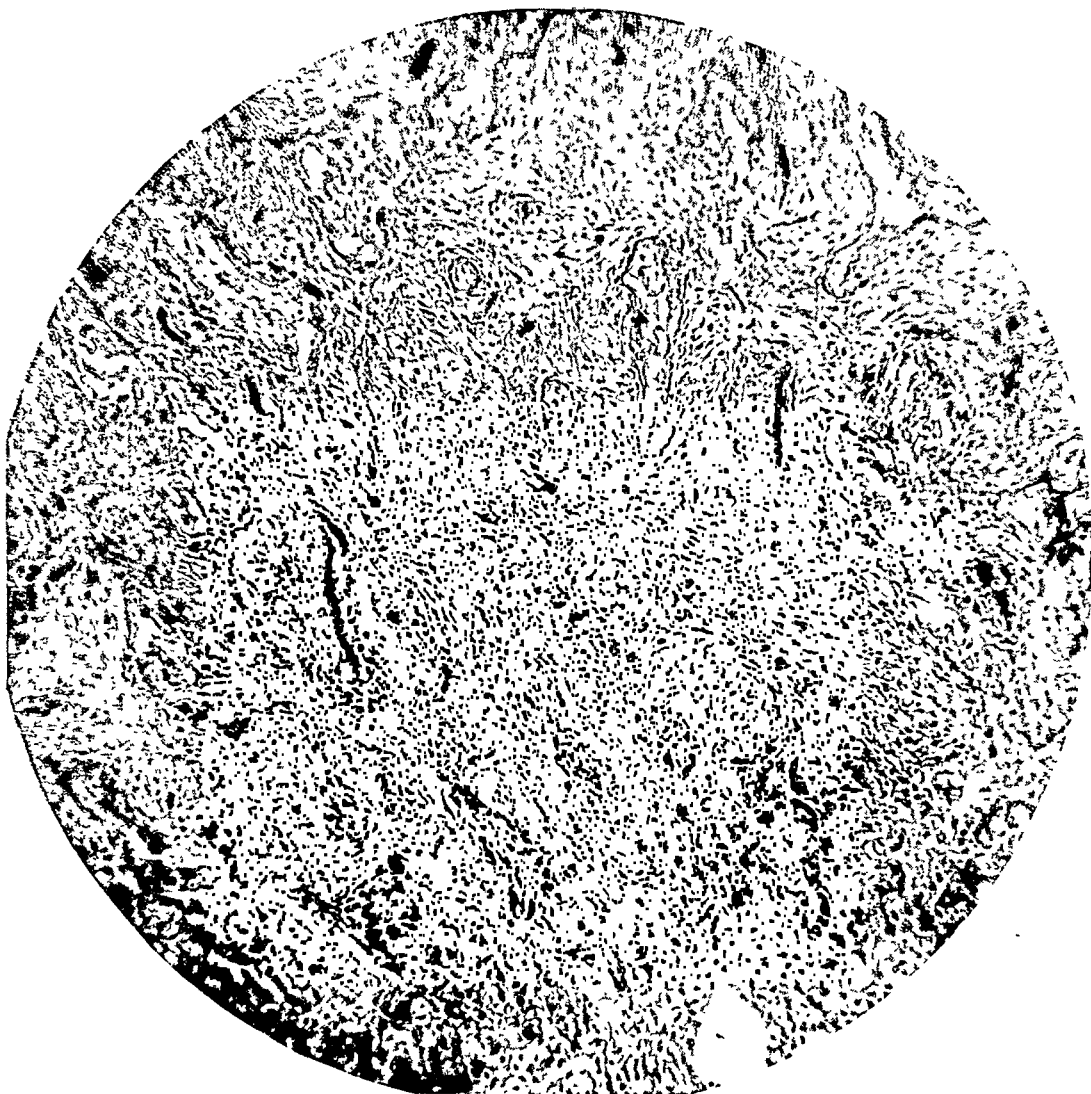


Fig. 3.—High power magnification of section shown in figure 2. Note epiphyseal cartilage in lower right corner, almost complete absence of bony trabeculae and fibrous tissue replacement of marrow.

essentially as severe in this animal as in control 22, which received parathormone alone.

Animals 24 and 25 received rapidly increasing doses of parathormone up to the twelfth day when 80 units were given. During the next three days the dosage was dropped to 10 units daily, at which level it was maintained until the end of the experiment on the twenty-sixth day (table). Animal 25 was given, in addition, 15 mg. of viosterol daily. The lesions of fibrous osteitis in this animal were much

more severe than those in animal 24, which received no viosterol. In fact, the changes in the bones of animal 24, which received parathormone alone, were slight, whereas the lesions in animal 25, especially those found in the long bones, were extensive. The occurrence of such slight lesions in the animal receiving parathormone alone might be explained on the basis of recent investigations published by Jaffe and his co-workers.<sup>13</sup> They suggested that in protracted experiments with

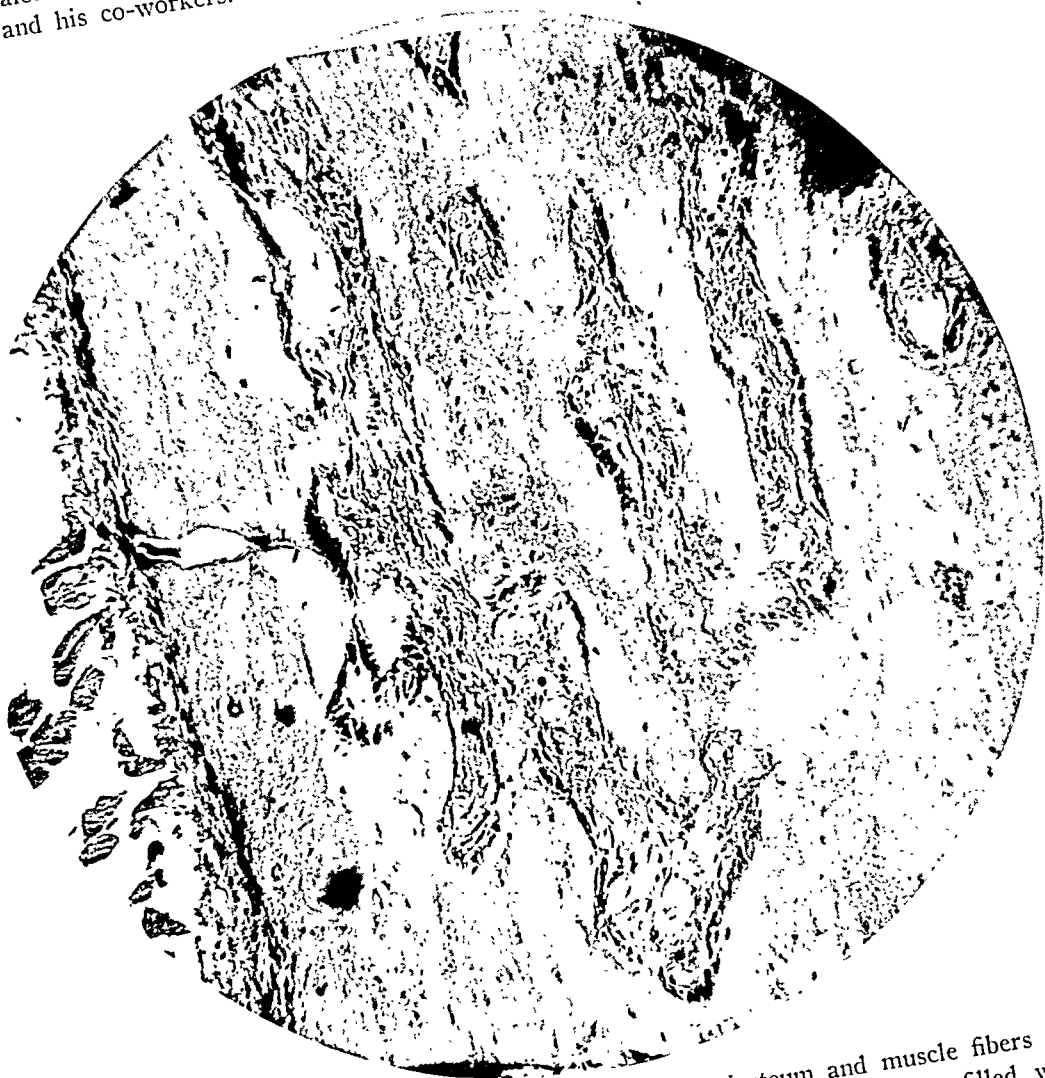


Fig. 4.—The cortex of a rib of animal 25. Periosteum and muscle fibers are seen on the left. The tremendously enlarged haversian canals are filled with vascular fibrous tissue.

parathormone on guinea-pigs a compensatory mechanism allows healing of lesions produced earlier in the treatment.

It is quite possible that this may have occurred in animal 24, which received only 10 units of parathormone daily during the last eleven days of the experiment.

13. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: *J. Exper. Med.* **55**:139, 1932.

Any compensatory mechanism that takes place evidently did not function so well in animal 25, which was given 15 mg. of viosterol daily. A synergistic action between parathormone and viosterol should possibly be considered in this instance.<sup>14</sup>

Animals 26 and 27 received increasing doses of parathormone up to a maximum of 80 units on the twelfth day, and no further injections during the remaining fourteen days of the experiment (table). Animal 27 was given 15 mg. of viosterol

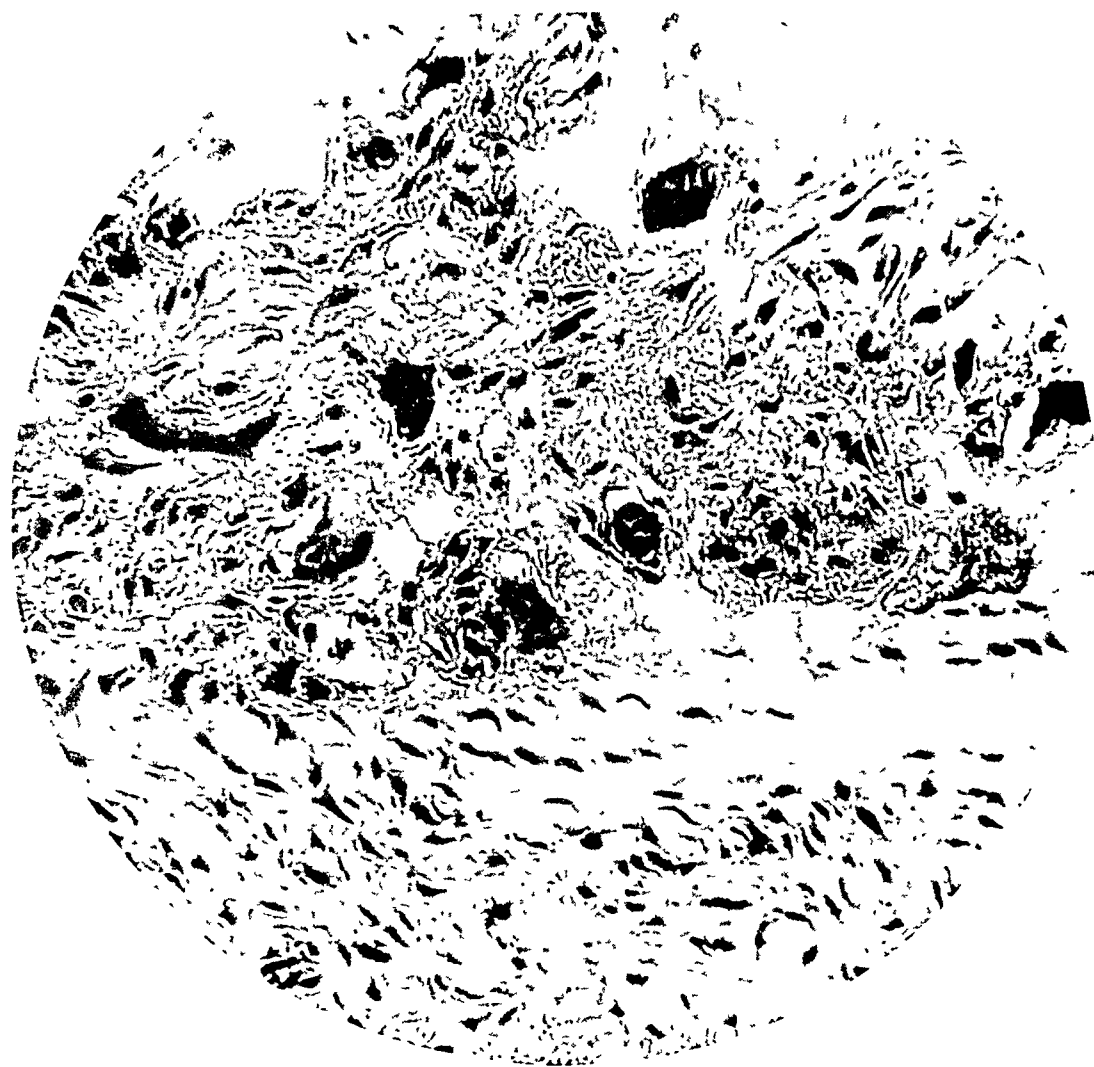


Fig. 5.—The metaphysis of a tibia of animal 27. An area of fibrous replacement containing many osteoclasts. Some of these are seen in Howship's lacunae.

daily, in addition. Both animals showed moderately severe lesions of fibrous osteitis, which were much more extensive in the long bones than in the ribs.

The table shows the dosage of parathormone given to animals 28 and 29. They received doses identical with those given the previous two pairs up to the fifteenth

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14. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: *Canad. M. A. J.* 25:1, 1931.

day, when the experiment was terminated. Animal 29 received 15 mg. of viosterol daily. Lesions of the severest grade were present in the ribs and long bones of no. 28. Animal 29, which received viosterol, showed lesions of fibrous osteitis of moderate severity, but in this case they were unquestionably less marked than those in animal 28 receiving parathormone alone.

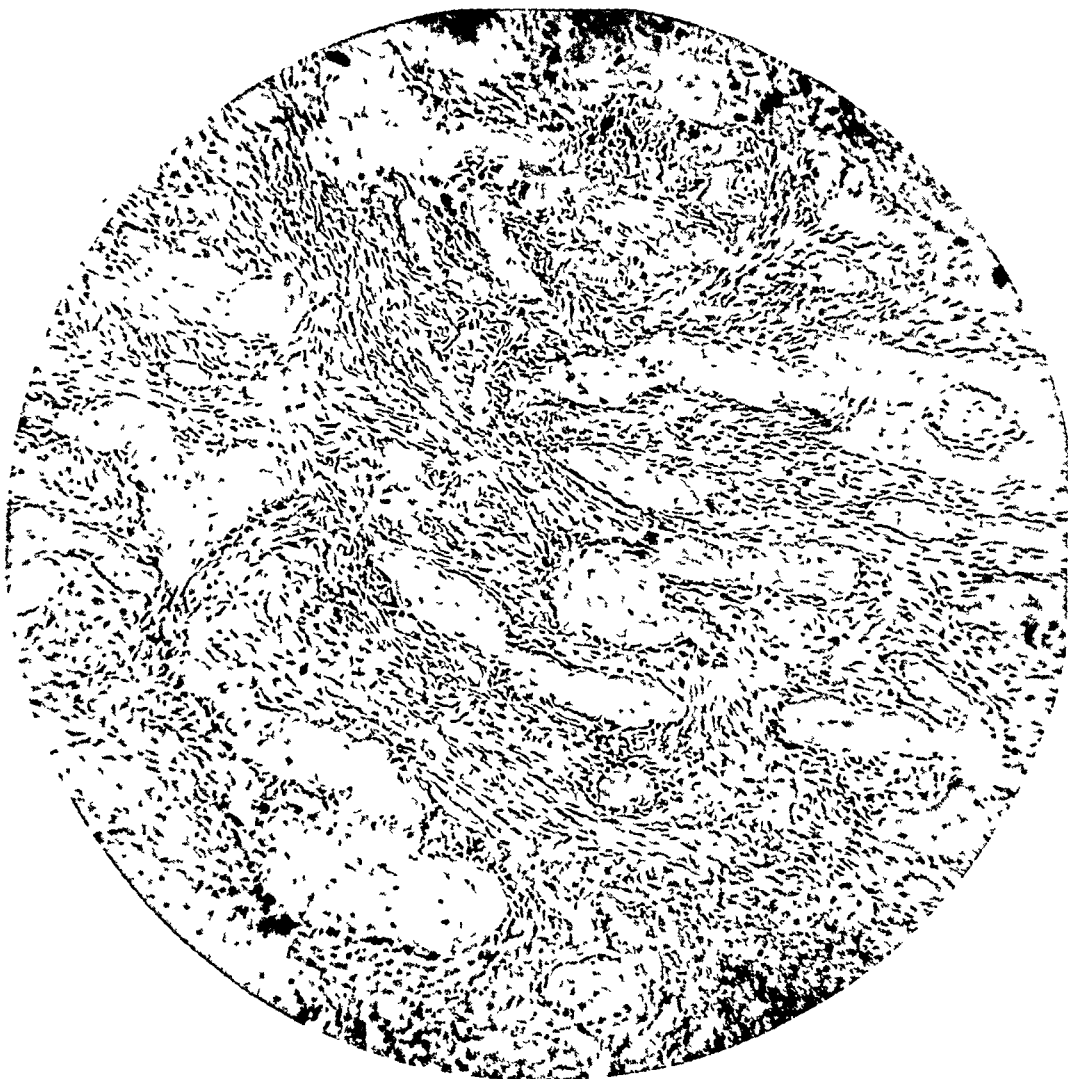


Fig. 6.—The cortex just below the costochondral junction of animal 28. Note the dense fibrous tissue surrounding the bony trabeculae.

#### SUMMARY

Three (7, 9 and 13) of five animals receiving viosterol plus 466 units of parathormone showed practically negligible changes in the bones compared with the control animals (4 and 20), which were given no viosterol. The lesions in the remaining two animals (18 and 21) were at least as severe as those found in the control. Two animals (28 and



29) were given rapidly increasing doses of parathormone over a period of fourteen days, the latter receiving viosterol. The lesions in this animal were strikingly less marked than those in its control. Throughout our work, it was only in this instance and in three of the animals of the previous group that viosterol seemed to show an antagonism to the action of parathormone.

Four animals (1, 19, 16 and 17) received 581 units of parathormone, the latter two being given viosterol. These two showed, if anything, even more severe fibrous osteitis than the two controls.

Two animals (5 and 15) received 849 units of parathormone. In the animal given viosterol (15) more extensive lesions were found than in the control.

Five animals (2, 3, 8, 12 and 14) received 326 units of parathormone. The lesions of the bones in all of them were comparatively mild, and were not significantly less in the three treated with viosterol (8, 12 and 14).

Animal 22 received rapidly increasing doses of parathormone, totaling 380 units. Equally severe lesions of the bones were found in this animal and in 23, to which viosterol was given.

In animal 24, which received 480 units of parathormone, the changes in the bones were comparatively slight. The lesions in animal 25, which received viosterol in addition, were severe.

Two animals (26 and 27) were given 310 units of parathormone over a period of twelve days, and then allowed to rest for fourteen days. Identical lesions of moderate severity were found in the bones of both animals in spite of the fact that viosterol was administered daily to animal 27.

#### COMMENT

Lesions of fibrous osteitis can be produced at will in guinea-pigs by repeated injections of parathormone. As the parathyroid hormone and vitamin D are antagonistic in several respects, an attempt was made to determine whether these lesions could be prevented by viosterol. The latter causes fixation of calcium in the bones, at least in rickets and osteomalacia, while the parathyroid hormone brings about mobilization of calcium from the bones. Viosterol produces a rise of serum phosphorus, whereas with parathormone a diminution is obtained. That both potent substances can raise the serum calcium, does not, as Hoff and Homann have remarked, mitigate against an antagonism. Parathormone increases the liberation of calcium from bone, whereas therapeutic doses of vitamin D increase the absorption of calcium from food.<sup>15</sup> In both instances, the blood may contain an increased amount of calcium. Since

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15. Bauer, W., and Marble, A.: *J. Clin. Investigation* **11**:21, 1932.

the hypercalcemia following overdosage with viosterol is probably dependent on demineralization of the skeleton,<sup>16</sup> small amounts of viosterol were given.

In this work, the lesions of fibrous osteitis were not prevented by viosterol. Although a few of the animals given viosterol showed less severe lesions, the changes in the bones of the great majority were unaffected. Jaffe and his co-workers reached the same conclusions.<sup>17</sup> Johnson and Wilder<sup>18</sup> apparently obtained essentially similar results, according to their preliminary report.

#### CONCLUSIONS

Repeated injections of parathormone produced the bone lesions of generalized fibrous osteitis in guinea-pigs.

Viosterol did not prevent these lesions.

An antagonism between vitamin D and the parathyroid hormone was not demonstrated by the method used in this work.

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16. Hess, A. F.; Benjamin, H. R., and Gross, J.: *J. Biol. Chem.* **94**:1, 1931.

17. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: *Proc. Soc. Exper. Biol. & Med.* **29**:202, 1931.

18. Johnson, J. L., and Wilder, R. M.: *Am. J. M. Sc.* **182**:800, 1931.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## IX. RESTORATION OF THE LIVER AFTER PARTIAL HEPATECTOMY AND PARTIAL LIGATION OF THE PORTAL VEIN

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Although it has been demonstrated<sup>1</sup> that restoration of the liver after either mechanical or toxic injury is actually restoration of the tissue, and that such recovery progresses more rapidly on a diet high in carbohydrates, only indefinite evidence has been published concerning the effect of alteration of the amount of blood reaching the liver by way of the portal vein.<sup>2</sup>

Following the report by Higgins and Anderson<sup>3</sup> on their success with partial hepatectomy in standardized white rats, it seemed that some application of their quantitative methods of investigation might be applied to the problem of the effect of partial ligation of the portal vein on restoration of the liver following partial removal.

### METHOD OF EXPERIMENT

In order to be sure of the ratio of the weight of the liver to the total weight of the animal, twenty-five rats from the stock pens were weighed and killed by exsanguination and their livers removed cleanly, rinsed lightly once, and weighed after light sponging to remove excess water. From the sum of the ratios of the weight of the liver to that of the body, a mean ratio of 0.0419 was derived. By applying the methods published by Dunn<sup>4</sup> in 1929, it was ascertained that this ratio was not only significant for all body weights within the limits of the group, but that there was a high correlation coefficient of 0.8726. From the same material it was possible to develop a formula by which to estimate the weight of the liver by the weight of the body of any given animal.

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Work done in the Division of Experimental Surgery and Pathology.

1. Fishback, F. C.: *Arch. Path.* **7**:955, 1929. Mann, F. C.: *Am. J. M. Sc.* **161**:37, 1921. Ponfick, E.: *Virchows Arch. f. path. Anat.* **118**:209, 1889. Rous, P., and Larrimore, L. D.: *J. Exper. Med.* **31**:609, 1920.

2. Mann, F. C.; Fishback, F. C.; Gay, J. G., and Green, G. F.: *Arch. Path.* **12**:787, 1931.

3. Higgins, C. M., and Anderson, R. M.: *Proc. Staff Meet., Mayo Clin.* **5**:243, 1930.

4. Dunn, H. L.: *Physiol. Rev.* **9**:275, 1929.

The finding that the relation of the weight of the liver to the weight of the body is practically constant, at least among the young adults that were observed, is amply borne out by Donaldson.<sup>5</sup>

After the establishment of standards, a series of rats was operated on according to the technic of Higgins and Anderson, with the additional procedure of partially occluding the portal vein by ligature as in the first stage of the operation described by Dragstedt.<sup>6</sup> After partial hepatectomy and weighing of the hepatic tissue removed, the portal vein was accurately identified, a point above the last tributary selected, and a linen ligature passed about it. This ligature was tied over a wire, which was then removed (fig. 1). The wire was so selected as to approximate half of the diameter of the vein. The abdominal wall was then closed in two layers. Following operation, the rats received

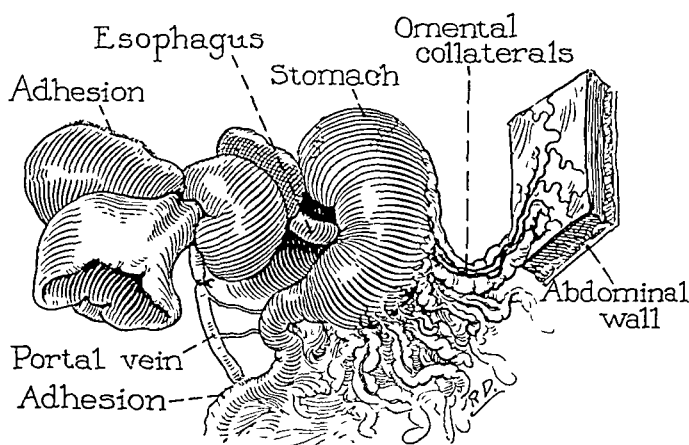


Fig. 1.—This semidiagrammatic view of the liver, stomach and omentum shows the operative procedure. Resection of the liver is completed, and the portal vein is partially ligated. The omental collateral vessels that develop following the operation and the small adhesions between the duodenum and the free edge of the liver are shown. These adhesions, separated for exposure, usually contain collateral vessels.

a solution of dextrose in water for the first twenty-four hours; they were then returned to the stock ration and tap water.

The mortality during the operation and in the succeeding twenty-four hours was rather high, as might be expected in such radical procedures on small animals. Necropsy was unsatisfactory except in the presence of peritonitis, which was unusual.

Groups of rats were killed at three, eight, fourteen, twenty-one and twenty-eight days after operation. These periods were selected as

5. Donaldson, H. H.: *The Rat: Data and Reference Tables for the Albino Rat*, Memoirs of the Wistar Institute of Anatomy and Biology, ed. 2, Philadelphia, 1924.

6. Dragstedt, L. H.: *Science* **73**:315, 1931.

most likely to reveal significant changes and as making it possible to compare the results with those of Higgins and Anderson. Each rat was lightly anesthetized and weighed, the abdomen opened, and the amount of collateral circulation determined. The animal was then killed by exsanguination, and the liver was dissected free from all attachments, rinsed once, dried lightly and weighed. Specimens were taken from typical livers in each group, and paraffin sections were stained with hematoxylin and eosin. These sections were compared with sections taken from the livers of normal rats used for the standard series.

After the expected weight of the liver had been calculated from the weight of the animal according to the formula derived from the standard series, it was possible to estimate the variation from the expected weight in each rat, and to determine the amount of change from the estimated weight of the remnant left at operation.

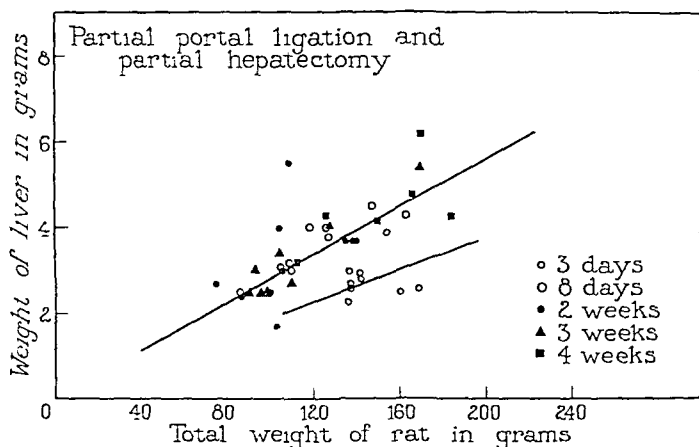


Fig. 2.—The graph shows the ratios after operation. In all cases, the weight of the liver was plotted against the weight of the body. The ratios cluster about one line, irrespective of the time interval, except for the first three days; in the three day group, the mean ratio is shown as a short line below the other. In each group, cases are well distributed as to weight of body.

#### OBSERVATIONS

Before operation, the ratio of the weight of the liver to that of the body was, as has been stated, 0.0419; the mean ratio of the remnants after operation was 0.0113 for the whole series. In eight rats killed on the third day after operation, the ratio was found to have increased to 0.0190. By the eighth day, in a group of ten rats, the ratio had further increased to 0.0289, but it changed little thereafter (figs. 2 and 3). After the eighth day, the curve of growth of the liver became almost a straight line at about 72 per cent of the expected ratio.

Considering the actual average weights of the liver in the various groups, there was a sharp rise in the mass of the remnants until the

eighth day, amounting to 2.38 Gm., after which there was a gradual fall again to 1.74 Gm. at four weeks. This represents an increase of about 300 per cent at the high point, but it is only about two thirds of that reported by Higgins and Anderson.

The effect of the operative procedure on the weight of the whole animal was definite and lasting. There was a decrease in total weight, starting immediately after operation, reaching its maximum at the second week, and returning toward normal after that time. However, at the end of the experiment, the average weight remained 5.98 Gm. below the preoperative level.

The response in restoration of the liver was correlated roughly with the amount of collateral circulation that had developed; it was evident

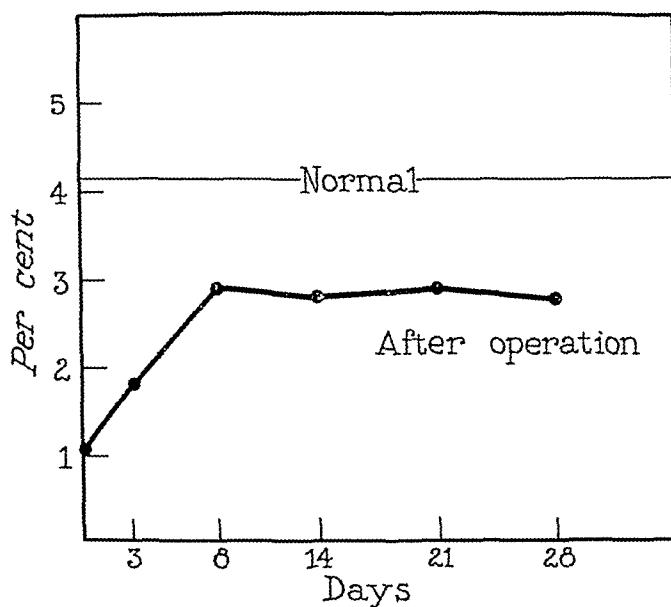


Fig. 3.—The graph shows the weight of liver after operation. The percentage of total weight was due to hepatic tissue at various periods after operation. After the eighth day, there was very little.

that in those rats with large collateral vessels restoration was definitely retarded. Likewise, large collateral vessels were coincident with loss of body weight. Conversely, the lack of collateral vessels was paralleled by increase in weight of liver, and in total weight as well. Thus, large collateral veins were taken as *prima facie* evidence of successful partial obliteration of the portal vein, and in such animals the ratio of the weight of the liver to the weight of the body was uniformly low.

The veins that developed after operation were usually most noticeable in the omentum, part of which was always found adherent to the abdominal wall at the operative scar (fig. 1). These collateral veins

were often larger than the normal portal vein, and were accompanied by smaller veins running up along the esophagus and in the adhesions usually found between the duodenum and the free edge of the liver.

In a few of the animals, the abdominal veins were grossly visible through the wall of the abdomen, but if the omental collateral vessels were large, transillumination disclosed many large vessels. Ascites was never found in an uncomplicated case, although occasionally at necropsy of an animal that had died from peritonitis, free fluid was found in the abdomen.

The cytologic changes were of interest during this period of restoration. In the normal rat, the capsule of the liver was usually thin, and the lobules were fairly large. Central lobular veins were about two or three for each low power field, and each lobule was surrounded by one or two distinguishable peripheral triads consisting of radicles of the portal vein, hepatic artery and biliary duct. The cells of the liver were regular, or polyhedral; they averaged about 20 microns in diameter, with a definite cell membrane and a homogeneous cytoplasm. The nuclei were about 8 microns in diameter, and each contained from one to three nucleoli.

On the third day after operation, the capsule of the liver was somewhat inflamed, being thicker and containing a few invading leukocytes. Central veins and peripheral triads were more prominent, averaging one more for each low power field than the normal, and the hepatic cells were larger, measuring about 24 microns. Their regular polyhedral outlines were lost, and the cytoplasm was definitely vacuolated. This was probably "cloudy swelling," for the most part, but there certainly was actual degeneration near the center of most of the lobules. The sinusoids were large and irregular near the central vein, but almost indistinguishable at the periphery of each lobule.

At the end of eight days, restoration had occurred to some extent, and conditions were almost normal. The cells near the central veins were irregular and had vesicular cytoplasm and small, deeply staining nuclei. The sinusoids were wider at the center of the lobule than at the periphery, but the picture might well be mistaken for the normal. These observations correspond with those reported by Milne.<sup>7</sup>

At two weeks and after, the only difference from the normal was in the vesicular character of the hepatic cells about the central veins. In the third and fourth weeks, some mitotic figures were seen in the parenchymal cells.

#### COMMENT

Although the results of these experiments seem to indicate that decreased portal blood supply may retard the restoration of the rat's liver after partial hepatectomy, it must not be assumed that this factor

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7. Milne, L. S.: *J. Path. & Bact.* **13**:127, 1909.

alone is responsible. It would strengthen such a conviction, however, if the problem were reversed and hypertrophy of the liver produced by establishment of active congestion through increased blood supply to the organ alone. This has not been done in rats because of technical difficulties, but Higgins<sup>8</sup> has mentioned that hypertrophy of the liver of a chicken was observed following ligation of the vena cava above the kidney, which caused a reversal of flow in the renal portal system and induced a distinct increase in the amount of portal blood reaching the liver.

Whether the amount of blood supplied to the liver through the hepatic artery bears any relation to the ability of the liver to regenerate following injury seems highly speculative. This has not been approached experimentally so far as is known, but this source of supply is so insignificant

*Averages and Ratios of Weight of Body and of Liver After Partial Hepatectomy and Partial Portal Ligation*

Group	Total Weight, Gm.	Weight of Liver at Operation, Gm.				Interval	Total Weight	Weight of liver When Animals Were Killed, Gm.				Total	Change of Weight of Liver			
		By Formula	Removed	Remaining				By Formula	Measured	Per Cent of Normal	Ratio		From Preop- erative Weight	From Postop- erative Weight	Per Cent	
				Grams	Ratio											
8	419.96	6.23	4.31	1.92	0.0127	3	146.57	6.07	2.80	46	0.0190	—	3.39	—3.44	0.88	146
10	126.38	5.03	3.88	1.15	0.0091	8	123.72	4.88	3.53	72	0.0289	—	2.66	—1.50	2.38	306
10	124.64	4.93	3.86	1.07	0.0085	14	110.06	4.20	3.19	76	0.0281	—	14.58	—1.74	2.07	296
8	120.10	4.73	3.46	1.27	0.0105	21	111.34	4.27	3.27	74	0.0288	—	8.76	—1.46	2.00	257
5	153.96	6.46	4.04	2.42	0.0157	28	147.98	6.14	4.16	67	0.0278	—	5.98	—2.30	1.74	171
41	135.00	5.47	3.90	1.56	0.0113		Averages									

nificant relatively that it does not prevent atrophy when the portal branches are ligated.

Qualitative studies of the portal blood in the liver following partial ligation of the portal vein have not been made, and I do not know how far certain hypothetic changes in the oxygenation of the tissues and decreased rates of flow may account for the results. However, it seems difficult to believe that any appreciable effect has been induced by such qualitative changes (if they exist), as the hepatic artery had supplied its full quota of blood to the organ.

Assuming that the amount of portal blood reaching the liver was mainly responsible for the reduced restoration following partial removal, how does that fact apply clinically? It may be that these observations explain, in part, the difference between the so-called portal cirrhosis and those degenerative processes that have been grouped together under the term acute atrophy. In both conditions, large quantities of hepatic

8. Higgins, G. M.: *Anat. Rec. (supp.)* 48:21, 1931.



tissue are affected, yet if death does not follow the acute destruction, the cirrhosis becomes progressive, and the atrophy usually ends by restoration, both physical and functional. In portal cirrhosis, the degenerative process is mainly about the portal radicles, and the fibrous tissue laid down in the process of repair must seriously impair the blood supply to the surrounding hepatic tissue, even from the first. In the presence of atrophy, however, the blood supply is adequate, and if the original injury has not been too great, and if the injuring agent is removed, complete restoration is the rule.

Observations of the effect of diverting part of the portal blood from the liver on the general weight of the body are of interest in the light of the clinical picture of hepatic infantilism, in which impaired development is explained on the basis of hepatic insufficiency. Although the livers in the rats seemed normal after operation, it is possible that the shunting of the blood through an artificial Eck fistula upset the bodily economy so that vital functions of growth were impeded.

#### SUMMARY

By combining partial hepatectomy with partial ligation of the portal vein in white rats, it has been possible to prevent, to some extent, the restoration that follows simple partial removal of the liver.

The livers of a series of forty-one rats studied over a period of four weeks did not return to more than 76 per cent of the expected weight, and the weights of the bodies of the animals were continuously below the preoperative level.

Although some of the degenerative and regenerative changes reported by others as following simple partial hepatectomy were observed, these changes were not so marked after this combined operative procedure.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## X. RESTORATION OF THE LIVER OF THE DOMESTIC FOWL

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AND

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The biologic factors that control the rapid restoration of the liver following partial removal of the organ are still imperfectly known. Restoration does not take place from the level of the cut surface, as in lower forms of life following removal of appendages, but results from cellular hypertrophy as well as hyperplasia of the lobes that remain following partial hepatectomy. Does the liver in the mammal recover its preoperative size and volume in response to a demand by the organism for functional hepatic parenchyma? This would appear probable, except for the fact that the volume of the liver in any normal animal is always functionally greater than that actually required. The liver, as shown clinically and pathologically, possesses a wide margin of safety, and there is every indication that an organism may function adequately with a greatly reduced hepatic parenchyma.

Experimentally Whipple and Hooper<sup>1</sup> showed that when in animals the portal blood was diverted from the liver through an Eck fistula into the posterior vena cava, the volume of the liver was reduced to half its normal size, and that, despite the reduction, with proper diet and housing, these animals maintained apparent good health. Mann and Magath<sup>2</sup> found that when 30 per cent of the liver of an animal with an Eck fistula was removed, the remaining portion remained unchanged, and there was essentially no restoration, and that even with this additional loss of hepatic parenchyma the animals remained apparently normal.

Stephenson<sup>3</sup> found that when he combined partial ligation of the portal vein with partial removal of the liver in the white rat, normal restoration of the organ did not occur. Although he attempted to con-

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1. Whipple, G. H., and Hooper, C. W.: *Am. J. Physiol.* **42**:544, 1917.

2. Mann, F. C., and Magath, T. B.: *Am. J. Physiol.* **59**:485, 1922.

3. Stephenson, G. W.: *Experimental Pathology of the Liver: IX. Restoration of the Liver Following Partial Hepatectomy and Partial Ligation of the Portal Vein*, *Arch. Path.*, this issue, page 484.

strict the portal vein in each animal to a corresponding diameter, it was obviously impossible to deliver equal amounts of blood to the hepatic remnant in every animal. Accordingly, varying amounts of hepatic tissue were restored, but he never encountered more than 76 per cent of the volume that, according to the work of Higgins and Anderson,<sup>4</sup> should develop in response to partial removal alone.

These observations following partial removal of the liver of the dog with Eck fistula, and of the rat after restricting the volume of portal blood entering the liver, indicate the significance of the blood volume as a factor in controlling the extent of hepatic restoration. If the volume of blood that entered a lobe of the liver largely determined the extent of restoration that occurred after partial removal of the organ, then, conversely, an increase of blood delivered to the hepatic remnant should induce an increased amount of new hepatic parenchyma. Accordingly we proposed to test that hypothesis.

The domestic fowl, because of certain vascular arrangements between the systemic and the portal circulation, is an excellent subject on which to determine the effect on the liver of increasing the amount of blood in the portal vein. There is an anastomosis between the inferior mesenteric vein and the iliac veins that lie along, or often embedded within, the kidneys. Thus, through the inferior mesenteric vein, a tributary of the portal vein, there is direct venous continuity between the portal and postcaval vascular systems.

The liver of the fowl is soft and friable in texture and ordinarily comprises two principal lobes, a right and a left, of which the right is considerably the larger. Although the two lobes are connected at their base by a narrow isthmus of hepatic tissue, the left lobe may be easily ligated and surgically removed and the liver thereby reduced to about 60 per cent of its original size. When the left lobe of the liver is removed, restoration of the remaining lobe, to an extent such as takes place in mammals, does not occur. In a large series of hens subjected to the removal of the left lobe of the liver slight restoration did occur, but we have never encountered a restored liver, even after two or three months, with a weight equal to its original preoperative weight. It is our opinion that under these conditions some of the portal blood may have been diverted through the anastomosis of the inferior mesenteric and iliac veins into the posterior vena cava and thereby the volume of portal blood that was delivered to the remaining right lobe of the liver reduced.

The data condensed in this report have been assembled from a series of observations that we have made on the livers of white leghorn cockerels in which we had diverted the blood in the iliac and the femoral

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4. Higgins, G. M., and Anderson, R. M.: *Arch. Path.* **12**:186, 1931.

veins from the posterior vena cava through the anastomosis with the inferior mesenteric vein into the portal vein and thence through the right lobe of the liver.

#### METHOD OF INVESTIGATION

Forty white leghorn cockerels, aged approximately 6 months and ranging in weight from 1,100 to 1,300 Gm., were used. The flock was divided into three groups, all of which were maintained on the same food and water ration and were housed in laboratory cages under identical laboratory conditions. Five fowls were not operated on, but served as the control group for data on the weight of the liver and the weight of the body during the period of observation. Ten fowls were subjected to partial hepatectomy, in which the left lobe of the liver was removed through a left lateral abdominal incision. Twenty-five fowls were subjected to a two stage operation wherein the posterior vena cava was ligated just anterior to the kidney at the first operation (fig. 1), and two or three weeks later the left lobe of the liver was removed.

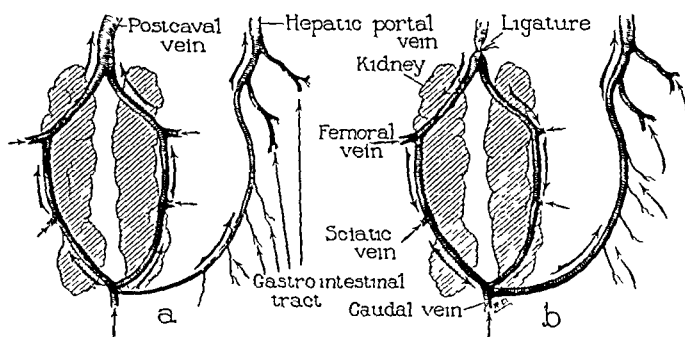


Fig. 1.—In *a*, normal vascular relations between the systemic and the portal circulation in the fowl are shown; in *b*, ligation of the postcaval vein and increased size of the portal system due to diversion of systemic blood.

Of the ten fowls from which the left lobe of the liver was removed, two died as a result of operative difficulties, whereas of the twenty-five subjected to the more difficult procedure of ligating the vena cava, three died during the operation, and nine others died from unknown causes during the ensuing twenty-four hours. Thus eight cockerels with only partial removal of the liver and thirteen with both partial removal and ligation of the posterior vena cava survived the operations, and the data derived from them with those from the control flock are reported. All cockerels were allowed to live for four months after operation and were killed by severing the blood vessels of the neck. All were healthy and had gained in weight; the weights ranged from 1,600 to 2,100 Gm. when the cockerels were killed.

#### OBSERVATIONS

Although the gross appearance of the restored livers indicated by the sizes of the remaining right lobes was sufficient to convince us that a difference in the extent of restoration had occurred in these two groups of cockerels on which operation had been performed, an analysis of the data from the standpoint of the weight of liver and body is difficult. It is obviously impossible to apply the statistical method, as we did with the

weights of the liver assembled from our study on hepatic restoration in white rats, for the number of fowls is insufficient. It is obviously impossible, too, to gain anything like an adequate estimate of the ratio of the weight of the liver to the weight of the body in this series. Furthermore, such a ratio at the time the fowls were killed would be quite different from that at the time of operation. In order to have accurate indication of the changes in the weight of the liver during the experimental period, it was necessary to know the weight of the liver in a large series of cockerels both at the time of operation and at the conclusion of the experiment. This we have not attempted. We have killed all fowls, however, at corresponding periods following a feeding, so that the changes in the weight of the liver, which pursues a cyclic activity related to feeding,<sup>5</sup> did not enter into our computation. The fowls were weighed just before killing. The livers were removed, all excess blood was drained from them, and they were carefully weighed.

Since the weights of these experimental cockerels when killed varied from 1,600 to 2,100 Gm., we have not attempted to correlate the weight of the liver with the weight of the body. But we have restricted consideration to the weight of the restored liver at the termination of the experiment, and have contrasted it with the estimated weight of the hepatic remnant after partial hepatectomy. Within certain limits of error we have computed the liver component that was removed at operation at 40 per cent of the total liver. This figure, we realize, is subject to error; for although the same lobe was removed in each animal, identical percentages of one lobe in relation to the total liver are not likely to obtain for all individuals. However, for our purposes, it has seemed adequate to estimate the amount of liver that remained within the fowl following partial removal on the basis of the amount removed. Thus, knowing the amount actually removed at operation, we were able to estimate the amount that remained within the peritoneum. Subsequently, when the fowl was killed four months later, the weight of the hepatic remnant subtracted from the final weight of the liver was the amount of liver accepted as the restored portion during the period of observation.

A summary of the data derived from all fowls subjected to partial hepatectomy, but without the ligation of the vena cava, showed that although a considerable amount of hepatic restoration had occurred, the preoperative weight of the liver was not regained. In this first series the average preoperative weight of the liver of the eight cockerels was estimated to be 28.25 Gm. An average of 11.30 Gm. was removed surgically from each fowl, so that 16.95 Gm. of liver was estimated as the average weight of the hepatic parenchyma that remained in the peri-

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5. Higgins, G. M.; Berkson, J., and Flock, E.: *Proc. Staff Meet., Mayo Clin.* 7:132, 1932.

toneum. At the end of the experiment, four months later, an average weight of liver of 24.67 Gm. was encountered. This is an amount 3.58 Gm. less than the estimated weight of the liver at the time of operation. Although the difference between these two figures is not large, yet in contrast with corresponding observations on mammals it is significant. Furthermore, there was an average increase of 550 Gm. in the weight of the cockerels during this period, so that certain increases in the weight of the liver might well have been anticipated. Some restoration had occurred, and the average increase in the weight of the liver during the four months over the estimated weight of the hepatic remnant following operation was 7.7 Gm., which is an increase of approximately 45 per cent.

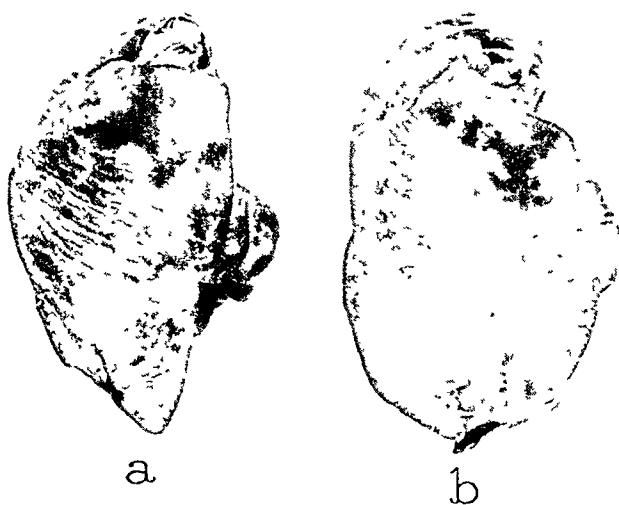


Fig. 2.—In *a* is shown the left lobe of the liver of a cockerel four months after removal of the right lobe; in *b*, left lobe of the liver of a cockerel four months after ligation of the postcaval vein and removal of the right lobe. The increase in size is apparent.

The data assembled from the group of thirteen cockerels in which the posterior vena cava was ligated and the left lobe of the liver removed showed a much greater increase in the extent of restoration of the remaining right lobe (fig. 2). In these cockerels, the inferior mesenteric vein was always from three to four times its usual size, distended by the increased volume of blood coursing from the legs and the kidneys to the portal vein. In this series, an average of 11.2 Gm. of liver was removed from ten days to two weeks after the posterior vena cava had been ligated, leaving an average of 16.78 Gm. of parenchyma within the cavity of the body. After four months, the cockerels were killed, and the average weight of the liver was 29.27 Gm. Since the hepatic remnant at operation was estimated to weigh 16.78 Gm., we conclude that approximately 12.49 Gm. of liver had been produced in each fowl

during the experimental period. In each of the thirteen successful experiments, a liver was encountered at necropsy greater in weight than that estimated present before operation. The average increase in the weight of the liver in these fowls subjected to both operations, in which the systemic blood from the legs and kidneys was shunted through the inferior mesenteric vein into the portal vein, was greater by 4.79 Gm. than that in the weight of the liver in those subjected to partial removal alone. In other words, as far as we were able to secure an accurate analysis of the data, we had induced in these fowls, by the increase in the amount of blood passing through the gland, restored livers approximately 62 per cent greater than in the flock with partial hepatectomy alone.



Fig. 3.—In *a* is shown the normal liver of a cockerel; in *b*, liver of a cockerel four months after diverting systemic blood into the portal system.

The relation between the size of the liver and the volume of blood traversing the organ is further indicated by a few observations that we have made on cockerels subjected to an operation in which the posterior vena cava above the kidney was securely ligated. In four fowls, successfully operated on and explored four months later, the livers were very much larger than when seen at operation and larger than those in the controls (fig. 3). It seems probable that the capillary bed in the liver in these fowls was inadequate to handle the increased volume of blood that was delivered to the portal vein, so that an increase in the size and volume of the gland resulted. We have not attempted to correlate the increase in the size of the gland with the increase in the amount of blood coursing through the portal vein, although no doubt the two bear a significant correlation.

There were no significant differences in the histologic appearance of these restored livers, and they were essentially like the livers from the

control fowls not operated on. We have not followed the changes that may have occurred in the right lobe of the liver immediately following the operative procedures, but we have studied sections taken from all fowls at the end of the experiment. There was no congestion in the liver that received the added volume of blood, and certainly sections stained with hematoxylin and eosin afforded no criteria that would render identification possible.

#### COMMENT AND SUMMARY

These results on fowl are wholly in accord with observations hitherto made on the dog and the rat, and substantiate rather definitely the opinion that the volume of blood which is delivered to any hepatic remnant after partial hepatectomy determines largely the extent of its restoration. It seems clear that the rapid restoration of a small portion of the liver to a size even greater than the normal organ, as in rats, is due to responses that are induced by mechanical factors associated with the volume of portal blood traversing the gland. In the cockerel, the degree of restoration after removal of the left lobe only was far less in proportion to that which occurs in the mammal, and it is our opinion that a considerable quantity of the blood that drains the gastro-intestinal tract passed through the anastomosis between the inferior mesenteric vein and the iliac veins to the posterior cava. When the systemic blood from the legs and back was delivered to the portal vein by ligation of the posterior vena cava just anterior to the kidney, the size of the remaining right lobe, after removal of the left, was greater in four months than the normal liver on which operation had not been performed.



# HEMATOPOIETIC EFFECT OF NUCLEAR EXTRACTIVES OBTAINED FROM RED BLOOD CELLS OF THE FOWL

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In 1926, we published <sup>1</sup> the observation that when nucleated red blood cells of the fowl (chicken and duck) were introduced into the blood stream of the rabbit, a marked increase in the number of the erythrocytes and in that of the leukocytes, as well as in the percentage of hemoglobin, occurred. The intravenous injection of washed nuclei from chicken blood cells produced the same effect; but the injection of the cell stroma—the hemoglobin-bearing portion of the cell—obtained from the horse and the dog did not produce this effect. The injection of nuclear extractives in the form of the sodium salts of nucleic acid and nucleoproteins obtained from chicken blood cells was followed by a like response.

In subsequent papers,<sup>2</sup> we reported experimental and clinical data on the effect of nuclear extractives obtained by the methods of Kossel-Neumann<sup>3</sup> and Hammarsten<sup>4</sup> from the liver, spleen, pancreas and heart of the beef, the thymus of the calf, the kidney, liver and stomach of the hog, and the liver of the salmon, when given by mouth to anemic dogs and to patients suffering from both primary and secondary forms of anemia. It was shown that an active hematopoietic response to the feeding of these substances took place within a few days, as shown by a rapid increase in the reticulocyte count, which was followed immediately by an increase in the erythrocyte count and in the hemoglobin content.

In the present paper, we give further data on the hematopoietic effect of nuclear extractives from chicken blood cells, obtained by the

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1. Arch. Path. **2**:698, 1926.

2. J. A. M. A. **89**:682, 1927.

3. Mathews, A. P.: *Physiological Chemistry*, ed. 5, New York, William Wood & Company, 1930, p. 169.

4. *Text Book of Physiological Chemistry*, ed. 6, edited by Mandel, New York, John Wiley & Sons, 1911.

aforementioned methods, when fed to dogs with controlled experimental anemia.

# METHODS

Anemia in which the hemoglobin content of the red cells was roughly 50 per cent, was produced and maintained in five dogs after the method of Whipple and Robscheit-Robbins.<sup>5</sup> On these anemic dogs, we conducted a series of eight experiments during a period of eighteen months in order to determine the amount of hematopoietic stimulation, if any, produced by the administration of nuclear extractives obtained from chicken blood cells. The authors mentioned had demonstrated

TABLE 1.—*Series 1, Dog 1*

Date	Weight, Kg.	Plasma Vol- ume, Cc	Blood Vol ume, Cc	Cell Vol- ume, Cc.	Hemo- globin, per Cent	Hemo- globin, Gm. per 100 Cc.	Red Blood Cells, Millions	Retic- ulo- cytes, per Cent	With drawn Blood, Cc.	With- drawn Hemoglo- bin, Gm.
1/12/30	30 0	479	874	395	....	14 8	5 76	..	..	6 6
1/26/30	30 0	576	952	376	..	14 2	5 77	..	..	5 1
2/ 2/30	30 0	..	..	..	82 6	14 4	..	..	246	34 0
2/ 9/30	26 5	..	..	..	74 3	10 25	..	..	130	13 3
2/16/30	26 0	..	..	..	77 4	10 68	..	..	268	28 4
2/23/30	27 0	..	..	..	66 0	9 11	..	..	320	29 2
3/ 2/30	26 0	..	..	..	54 6	7 54	..	..	116	9 8
3/ 9/30	24 0	..	..	..	45 7	6 31	..	..	10	0 6
3/16/30	23 0	447	680	233	49 6	6 83	3 53	..	46	3 07
3/23/30*	23 0	..	..	..	44 4	6 10	3 91	..	10	0 6
3/30/30	22 2	..	..	..	71 4	9 86	5 42	..	200	19 7
4/ 7/30	23 4	..	715	..	85 4	10 11	5 80	..	234	23 4
4/13/30	22 2	..	..	..	78 4	8 56	5 74	..	185	15 3
4/20/30	22 7	..	664	..	89 8	9 81	..	..	160	14 2
4/27/30	23 5	448	640	192	72 3	9 9	4 92	..	200	19 2
5/ 3/30	24 0	..	690	..	72 7	10 04	4 68	..	200	20 08
5/11/30	24 2	..	..	..	58 4	8 03	..	1 0	100	8 03
5/18/30	24 2	..	..	..	61 7	8 50	4 79	0 6	10	0 85
5/25/30*	24 2	..	..	..	61 6	8 5	4 00	0 2	150	12 04
6/ 1/30	24 5	..	..	..	51 2	7 06	5 20	6 0	10	0 76
6/ 7/30	25 2	(Dog died)	..	..	74 6	10 3	6 52	5 6	200	21 0

\* Ten grams of nuclear extractives was added to the diet from March 23 to April 20 and from May 25 to June 7, 1930.

in their experimental work that the amount of hemoglobin regenerated in the body can be modified at will by control of the diet. We verified this conclusion in our experiments. In order to insure a constant stimulus to the production of hemoglobin and red blood cells, we maintained for these animals throughout a routine diet that contained ample quantities of all the food elements essential for normal growth, nutrition and health with a minimum of blood-forming materials.<sup>5</sup> By means of a series of frequent bleedings of calculated amounts we maintained a severe secondary anemia with a hemoglobin level about 50 per cent of normal. The samples of blood removed were measured each time for total hemoglobin, and we estimated from week to week the amount of hemoglobin each animal could produce under the standard

5. Am. J. Physiol. 72:395, 1925.

controlled conditions of diet, room temperature, cleanliness, contentment and exercise. We assumed that under these conditions, with a hemoglobin level kept at approximately 50 per cent of normal, the stimulus to hemoglobin and red blood cell formation was a sustained maximum, and therefore should test the value of any diet or therapeutic agent used for hematopoietic stimulation.

The dog was chosen as an experimental animal because its hematopoietic system is relatively stable and because it is large, easily trained, and friendly (factors that aid in maintaining maximum accuracy of technic) and because it is a good feeder, is hardy and keeps healthy despite prolonged severe anemia. Each dog was kept in a separate, well

TABLE 2.—*Series 1, Dog 2*

Date	Weight, Kg.	Plasma Vol-ume, Cc.	Blood Vol-ume, Cc.	Cell Vol-ume, Cc.	Hemo-globin, per Cent	Hemo-globin, Gm. per 100 Cc.	Red Blood Cells, Millions	Retic-ulo-cytes, per Cent	With-drawn Blood, Cc.	With-drawn Hemoglo-bin, Gm.
1/12/30	22	406	815	409	131.6	18.15	5.86	...	105	18.16
3/16/30	24	555	875	320	72.5	7.35	4.85	...	230	16.90
4/ 6/30	23	...	765	...	74.6	8.8	....	...	148	16.3
4/13/30	23	...	...	...	72.0	9.95	4.39	...	234	23.3
4/20/30	25	...	663	...	69.0	9.52	....	...	155	13.0
4/27/30*	26	575	736	161	52.0	7.13	3.98	0.8	43	3.1
5/ 4/30	27	...	...	...	58.8	8.25	5.34	5.0	8	0.7
5/11/30	28	692	932	240	61.0	8.42	....	...	60	5.05
5/18/30	28	...	...	...	81.6	11.26	7.46	...	258	29.0
5/25/30	28	...	...	...	75.1	10.35	5.2	1.2	233	24.1
6/ 1/30	28	...	...	...	52.8	7.29	4.9	...	8	0.6
6/16/30	30	...	...	...	70.0	9.2	5.2	0.8	92	8.5
6/23/30*	29	522	710	188	60.0	8.2	4.2	0.5	8	0.6
6/26/30	..	...	...	...	....	....	5.06	6.0	...	....
6/29/30	..	...	...	...	80.5	11.6	7.10	4.6	157	18.5

\* Ten grams of nuclear extractives was added to the diet from April 27 to May 18 and from June 23 to June 29.

ventilated, warm, clean compartment of the kennel. It was fed a weighed amount of the standard diet once daily, was given water at all times and was exercised daily.

After the dogs were accustomed to their surroundings, we determined in each one the normal blood plasma volume and cell volume, the red blood cell count, the percentage of reticulocytes, the percentage of hemoglobin, the grams of hemoglobin per hundred cubic centimeters of blood and the body weight. These normal calculations were checked and rechecked before inducing anemia. The blood volumes were obtained by the method of Hooper, Smith, Belt and Whipple.<sup>6</sup> Our results checked well, and we became satisfied with the accuracy of the method. For the determination of the percentage of hemoglobin and the grams of hemoglobin per hundred cubic centimeters of blood, we

6. Am. J. Physiol. 51:205, 1920.

followed the method of Osgood and Haskins.<sup>7</sup> Twenty estimations by this method on one sample of blood, each estimation being checked by at least two observers, gave a percentage of error of not to exceed 1 per cent. The red blood cell counts were made with oxalated venous blood,

TABLE 3.—*Series 1, Dog 3*

Date	Weight, Kg.	Plasma Volume, Cc.	Blood Volume, Cc.	Cell Volume, Cc.	Hemoglobin, per Cent	Hemoglobin, Gm. per 100 Cc.	Red Blood Cells, Millions	Reticulo-cytes, per Cent	With-drawn Blood, Cc.	With-drawn Hemoglobin, Gm.
1/12/30	21.0	463	891	428	91.6	15.9	5.76	...	...	...
1/26/30	25.0	...	...	...	...	14.4	6.02	...	45	5.14
2/ 9/30	26.0	...	...	...	96.7	13.36	...	...	300	40.5
2/16/30	28.0	...	...	...	86.2	11.9	...	...	126	14.9
2/23/30	24.0	...	...	...	59.9	8.25	...	...	220	18.2
3/ 2/30	24.0	...	...	...	59.8	8.25	...	...	200	16.5
3/ 9/30	23.7	...	...	...	60.3	8.32	4.05	...	205	17.1
3/16/30	24.0	366	526	160	56.9	7.85	3.38	...	190	14.3
3/23/30*	23.0	...	...	...	49.3	6.8	3.18	...	...	0.7
3/30/30	22.5	...	...	...	97.6	13.46	5.86	10.3	260	33.2
4/ 7/30	23.5	...	825	...	84.5	12.42	4.64	...	266	27.8
4/13/30	24.0	...	706	...	69.4	8.42	...	...	150	12.55
4/20/30	24.0	...	860	...	64.7	7.86	3.32	...	100	7.86
4/27/30	24.0	564	693	129	...	7.92	3.5	...	50	3.5
5/ 4/30	25.0	613	779	166	...	...	3.88	0.8	...	...

\* Ten grams of nuclear extractives was added to the diet from March 23 to April 7, 1930.

TABLE 4.—*Series 2, Dog 1*

Date	Weight, Kg.	Plasma Volume, Cc.	Blood Volume, Cc.	Cell Volume, Cc.	Hemoglobin, per Cent	Hemoglobin, Gm. per 100 Cc.	Red Blood Cells, Millions	Reticulo-cytes, per Cent	With-drawn Blood, Cc.	With-drawn Hemoglobin, Gm.
12/ 4/30	30.5	...	...	...	115.3	15.9	6.84	...	62	9.9
12/11/30	29.5	590	1090	500	106.0	14.6	6.25	...	287	42.0
12/16/30	27.0	548	880	332	89.6	12.4	4.45	...	231	30.3
12/30/30	27.5	...	...	...	78.2	10.7	...	...	283	30.5
1/ 1/31	27.0	...	...	...	86.4	11.9	...	...	150	17.9
1/ 8/31	25.0	...	...	...	93.3	12.9	...	...	295	38.0
1/13/31	28.0	...	...	...	78.35	10.8	...	...	218	23.6
1/20/31	27.5	...	...	...	82.7	11.4	...	1.7	295	33.6
1/27/31	27.5	...	...	...	87.9	12.1	4.51	...	274	33.2
2/ 3/31	27.5	...	750	...	71.8	9.9	4.15	...	228	22.6
2/10/31	28.0	...	...	...	69.1	9.5	2.94	...	175	16.67
2/17/31*	28.0	...	...	...	50.3	6.9	2.99	0.9	50	3.5
2/24/31	27.0	...	...	...	78.9	10.84	5.13	...	240	25.2
3/ 3/31	28.5	451	670	119	83.8	11.55	6.1	15.0	207	24.0
3/10/31	28.5	...	...	...	78.9	10.9	5.90	...	215	23.4
3/17/31	27.5	...	...	...	73.35	10.1	5.7	8.0	240	24.2
3/24/31	28.0	...	...	...	91.3	12.6	8.62	...	318	41.0
4/ 5/31	28.5	...	...	...	72.4	10.0	...	...	215	21.5

\* Ten grams of nuclear extractives was added to the diet from Feb. 17 to March 19, 1931.

with the standard technic and the improved Neubauer hematocytometer calibrated for research work. The percentage of reticulocytes and the total number of reticulated red blood cells per cubic millimeter of blood were obtained by using brilliant cresyl blue and a light counterstaining with Wright's stain.

7. Osgood, E. E., and Haskins, H. D.: A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students, P. Blakiston's Son & Co., 1931, p. 347.

The dogs were bled once each week. Before each bleeding we estimated the blood volume of the dog and the grams of hemoglobin per hundred cubic centimeters of blood, from which we calculated the amount of hemoglobin necessary to be removed in order that the total hemoglobin in grams left in each animal should equal 50 per cent of the original normal. Sufficient blood was then removed to leave the animal with one half of its original hemoglobin as estimated in grams. Never, however, did we remove more than one third of the total volume of blood, and usually three or four bleedings were necessary to reach a 50 per cent hemoglobin level. For approximately two months the

TABLE 5.—*Series 2, Dog 2*

Date	Weight, Kg.	Plasma Vol- ume, Cc.	Blood Vol- ume, Cc.	Cell Vol- ume, Cc.	Hemo- globin, per Cent	Hemo- globin, Gm. per 100 Cc.	Red Blood Cells, Millions per Cent	Retic- ulo- cytes, per Cent	With- drawn Blood, Cc.	With- drawn Hemoglo- bin, Gm.
12/16/30	29.0	...	...	...	116.0	16.05	6.75	...	35	5.6
12/20/30	27.5	604	1160	556	107.7	14.8	....	...	318	47.2
1/ 1/31	28.0	...	...	...	85.8	11.85	....	...	285	33.7
1/ 8/31	25.5	...	...	...	93.0	12.90	....	...	290	37.3
1/13/31	30.0	...	...	...	75.7	10.4	....	...	218	28.9
1/20/31	28.5	...	...	...	72.8	10.1	....	...	318	32.0
1/27/31	27.5	...	...	...	80.4	11.1	3.82	...	281	31.8
2/ 3/31	26.5	...	834	...	66.9	9.10	3.48	...	165	16.7
2/10/31	25.0	...	...	...	69.0	9.60	3.05	...	100	9.6
2/17/31*	24.5	...	...	...	58.10	8.00	3.51	...	40	3.2
2/24/31	24.5	...	...	...	84.90	11.70	5.92	1.1	240	28.1
3/ 3/31	25.0	...	...	...	69.10	9.50	5.40	4.0	260	24.8
3/10/31	26.5	500	750	250	69.9	9.65	6.40	...	250	24.2
3/17/31	26.5	...	...	...	73.6	10.2	5.90	...	258	26.2
3/24/31	25.5	...	...	...	77.0	10.6	6.55	1.2	270	27.7
4/ 7/31	25.0	...	...	...	74.3	10.2	....	...	285	29.4
4/21/31	25.0	...	...	...	77.0	10.2	....	...	235	25.0
4/29/31*	25.0	550	755	196	78.3	10.8	3.85	...	200	21.6
5/ 5/31	27.0	...	...	...	84.6	11.7	6.09	5.5	300	42.0
5/15/31	28.0	...	...	...	96.2	13.3	....	...	325	43.2
5/19/31	28.4	...	...	...	73.6	10.25	6.11	7.1	335	34.34

\* Ten grams of nuclear extractives was added to the diet from Feb. 17 to March 10 and from April 29 to May 19, 1931.

weekly regeneration of hemoglobin was astonishingly great; it often equaled one fourth of the amount of the original total hemoglobin. With each bleeding the regenerated hemoglobin, however, became less, so that after from ten to twelve weeks it remained comparatively small. We were obtaining, therefore, at this point the maximum stimulus to the production of hemoglobin from the diet; all the reserve hematopoietic materials had been exhausted by the repeated losses of blood.

The different animals responded differently to the bleedings, both as to regeneration of hemoglobin and as to the time needed to deplete the body of reserve hematopoietic materials, but on the whole the ultimate results were satisfactory. After maintaining a constantly severe secondary anemia with the maximum hematopoietic stimulation possible from the diet used and with a minimum amount of hemoglobin

regenerated weekly, we added to the diet daily 10 Gm. of the nuclear extractives obtained from the red corpuscles of chicken blood.

## OBSERVATIONS

When the regeneration of hemoglobin was less than 6 Gm. per week, we considered the anemic animal satisfactory for experimental use. The average total amount of hemoglobin in our normal dogs was 152 Gm., with a low content of 146 Gm. and a high content of 162. The total content of hemoglobin in the anemic dogs was between 50 and 60 Gm. After the addition of 10 Gm. of nuclear extractives daily to the diet, the dogs regenerated from 23 to 29 Gm. of hemoglobin each week, whereas, on the standard diet alone, they regenerated less than 6 Gm. in

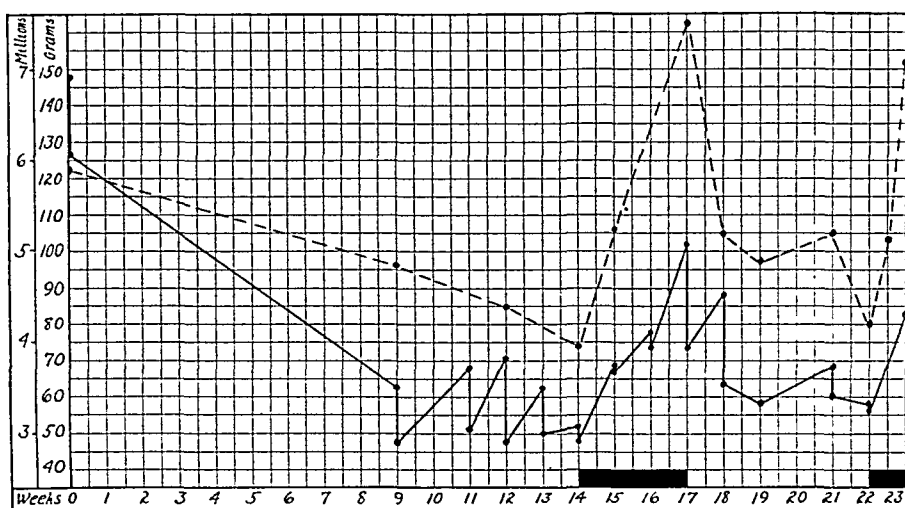


Chart 1.—The solid line represents estimated total hemoglobin in grams, based on percentage of hemoglobin and grams of hemoglobin per hundred cubic centimeters of blood at the time of the different readings. The horizontal continuation of this line represents time in weeks (two spaces per week). The vertical variation represents changes in the amount of hemoglobin. The vertical sections of the line between two points of the same week represent withdrawals of blood, one space representing 5 Gm. of hemoglobin. The broken line represents the red blood cell count. The weekly counts made during the preliminary stages of bleeding are not indicated, giving the unbroken descent of this portion of the graph. Red blood cell counts after the amount of hemoglobin was sufficiently reduced are indicated weekly with the estimations of hemoglobin. The heavy blocks of black indicate periods during which nuclear extractive was added, in the amount of 10 Gm. daily, to the regular diet. In chart 1, the hemoglobin curve and the curve of the red blood cell count of dog 2, series 1, are shown.

the same period of time. We noted, too, that for three or four weeks following the withdrawal of the nuclear extractives from the diet the dogs continued to regenerate from 18 to 20 Gm. of hemoglobin each week, indicating not only that the extractives were capable of stimulat-

ing an immediate production of hemoglobin, but also that an amount of the stimulant was stored in the animal's body sufficient to maintain regeneration for from three to four weeks longer. We noted further that if the extractives were again administered before depletion of the stimulant had occurred, the animal responded at once by a greater production of hemoglobin than had taken place in the beginning. For example, dog 2, series 2, regenerated 29, 25 and 21 Gm. of hemoglobin, respectively, during three successive weeks following the omission of the extractives from the diet, and during the fourth week, on 10 Gm. of the extractives daily, regenerated 42 Gm. of hemoglobin. The accompanying tables give the details of the experiments.

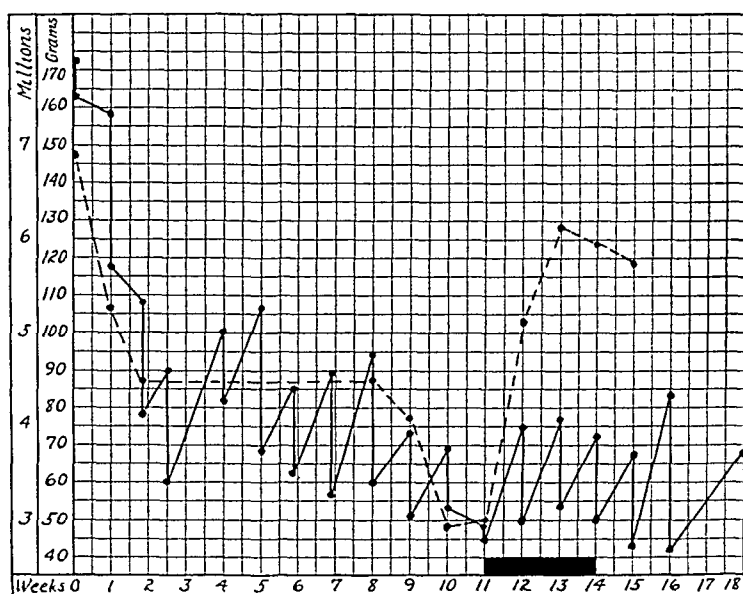


Chart 2.—The hemoglobin curve and the curve of the red blood cell count of dog 1, series 2. See legend of chart 1 for further explanation.

#### COMMENT

The problem of the effect of nuclear extractives obtained from the red blood cells of the fowl began in 1924 as a histologic study of the fate of the whole blood cells when introduced into the blood stream of the rabbit. The animals surviving the initial shock of the blood transfusion showed uniformly profound hematopoietic stimulation. The red blood cell count increased in certain instances from 32 to 55 per cent above the normal and remained elevated for periods of weeks. It was found that the stroma of the cell did not produce this effect, but that washed nuclei obtained from the cells did, and that nucleoproteins and sodium salts of nucleic acids possessed the same properties. Administration of the latter extractives was made at first by intravenous

injection, both into normal rabbits and dogs and into patients suffering from pernicious anemia and different forms of secondary anemia. Hematopoietic stimulation was observed in both instances, but the patients frequently suffered anaphylactic-like shock, possibly from lack of refinement of the product, and this method was therefore replaced by oral feeding. The making of the extractives from chicken blood cells in sufficient quantity to continue experimentation proved to be impractical, and because the unknown factor causing the hematopoietic effect seemed to be confined to the nucleus, extractives were obtained by the same methods from the various glandular organs mentioned. These extractives were studied in the same manner as those from the chicken blood cells, with the result that the same hematopoietic effect was obtained in each instance, and this effect seemed to be roughly proportional to the amount of nuclear extractives in the several organs.

As many factors may enter into the composition of animal fractions obtained from liver, kidney, stomach, etc., the present study of extractives from the red blood cells of the fowl, from which extraneous factors were automatically eliminated, was planned. A sufficient quantity of the material was prepared to permit the experimental work described and also to treat clinically a few patients with pernicious anemia. Thus far three such patients have been treated for a sufficient length of time to recognize the same response in reticulocyte count, red blood cell count and hemoglobin content that follows the proper administration of liver or stomach extractives available in the market. The records of these patients will be published elsewhere.

#### SUMMARY

The hematopoietic response in a series of five dogs with severe anemia, produced by periodic bleedings and controlled by a standard diet, to the daily oral administration of 10 Gm. of nuclear extractives from the nucleated red blood cells of the fowl has been studied eight times.

The response to the feeding of these extractives consists of a sharp rise in the percentage of reticulated red blood cells, an increase in the red blood cell count and a corresponding increase in the percentage and volume of the hemoglobin content.

As no other factors that may influence regeneration of the blood have entered into these controlled experiments, it is permissible to conclude that a factor exists within the nucleus of the red blood cell of the fowl which has the power to stimulate markedly the production of blood in the dog.

From these results and from other experimental work previously reported by us it is reasonable to conclude that this hematopoietic stimulant is common to the nuclei of many tissue cells.



# Laboratory Methods and Technical Notes

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## AN AUTOPSY TABLE—A NEW DESIGN

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If one is confronted by the problem of equipping an autopsy room, two views of the situation may be taken. One may look on it as just another equipment problem the solution of which is to be found in the catalogue of any one of a number of manufacturers merely by turning to the index, a problem requiring little thought and deserving less, since, after all, it may be said that essentially one needs only a cramped cellar room, a pair of carpenter's horses and two boards, a pair of scissors, a saw and a butcher's knife. On the other hand, the problem may be considered worthy of some deliberation, especially since the autopsy room can be made a place where more than pathologic anatomy may be taught.

I have found justification for the second attitude toward the problem in the following reasonably sensible propositions.

First of all, good autopsy technic is a *sine qua non* of postmortem investigation of disease. Without this, the procedure is of little value and in a large number of instances actually harmful, since poor technic reflects discredit on a fundamentally unassailable method of investigation of disease.

In the second place, good technic cannot be taught or insisted on without at least reasonably convenient equipment.

The third proposition is that all clinicians are by nature repelled (and not infrequently justifiably so) by the autopsy, which they look on as a ghastly procedure that they are more than happy to leave to the pathologist, in spite of the element of necessity for the common good which they recognize in the procedure.

The fourth and last proposition is, therefore, that it is to the distinct advantage of all parties concerned that the autopsy be done in the neatest, cleanest manner, and that everything done shall be with due regard for common sensibilities. The autopsy room should, therefore, be so located and so equipped that it will attract and not repel.

Thus guided, I have given some thought to the planning of an autopsy table. To a large degree I have been forced to do this by the obvious inadequacies of the various tables offered by the manufacturers, both domestic and foreign, and by the desire to conserve the equipment funds of my department. The table which I have designed has been in use for about two years and has proved eminently satisfactory from every essential point of view. It was constructed partly by manufacturers in Baltimore and partly in the shop of Duke University. The details and specifications are as follows:

## SPECIFICATIONS FOR AUTOPSY TABLE

*Table Top*—1 The top (figs 1 and 2) is made from a slab of white marble 4 by 30 by 94 inches (10.16 by 76.20 by 238.76 cm.).

2. The surface is countersunk according to the measurements given in figure 3 *B*, allowing for a rim or margin of  $1\frac{1}{2}$  inches (3.77 cm.) of thickness.

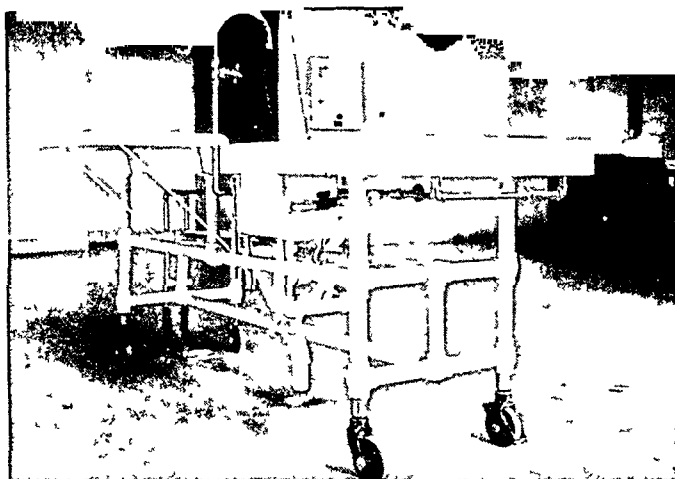


Fig. 1.—Autopsy table, showing the small sink, the valves in the water supply lines and the detachable side table.

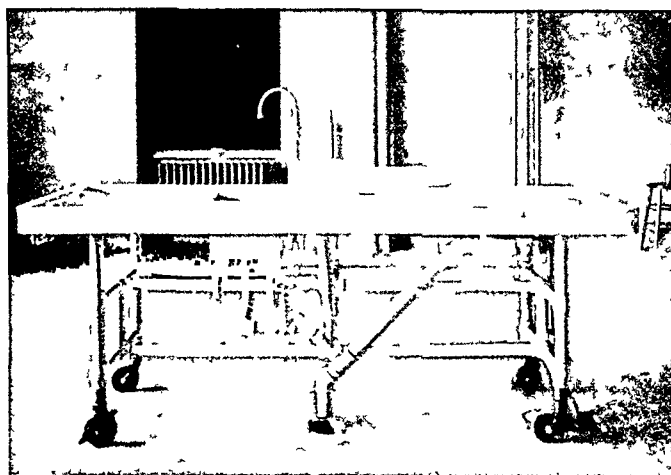


Fig. 2.—Autopsy table, showing the plan of the marble top.

3. The surface is graded in such a way that all drainage falls toward the main drain pipe near the foot of the table, as shown in figure 3 *B*. From the head to the main drain there should be a fall of at least  $\frac{3}{4}$  inch (1.83 cm.). In addition to this there should be a fall from the extreme sides to the center (fig 3 *C*) of at least  $\frac{1}{4}$  inch (0.61 cm.).

4. As will be seen in figure 3 *B*, the foot of the table consists of a shelf, the surface of which is 1 inch (2.5 cm.) above the surface of the table at the line of

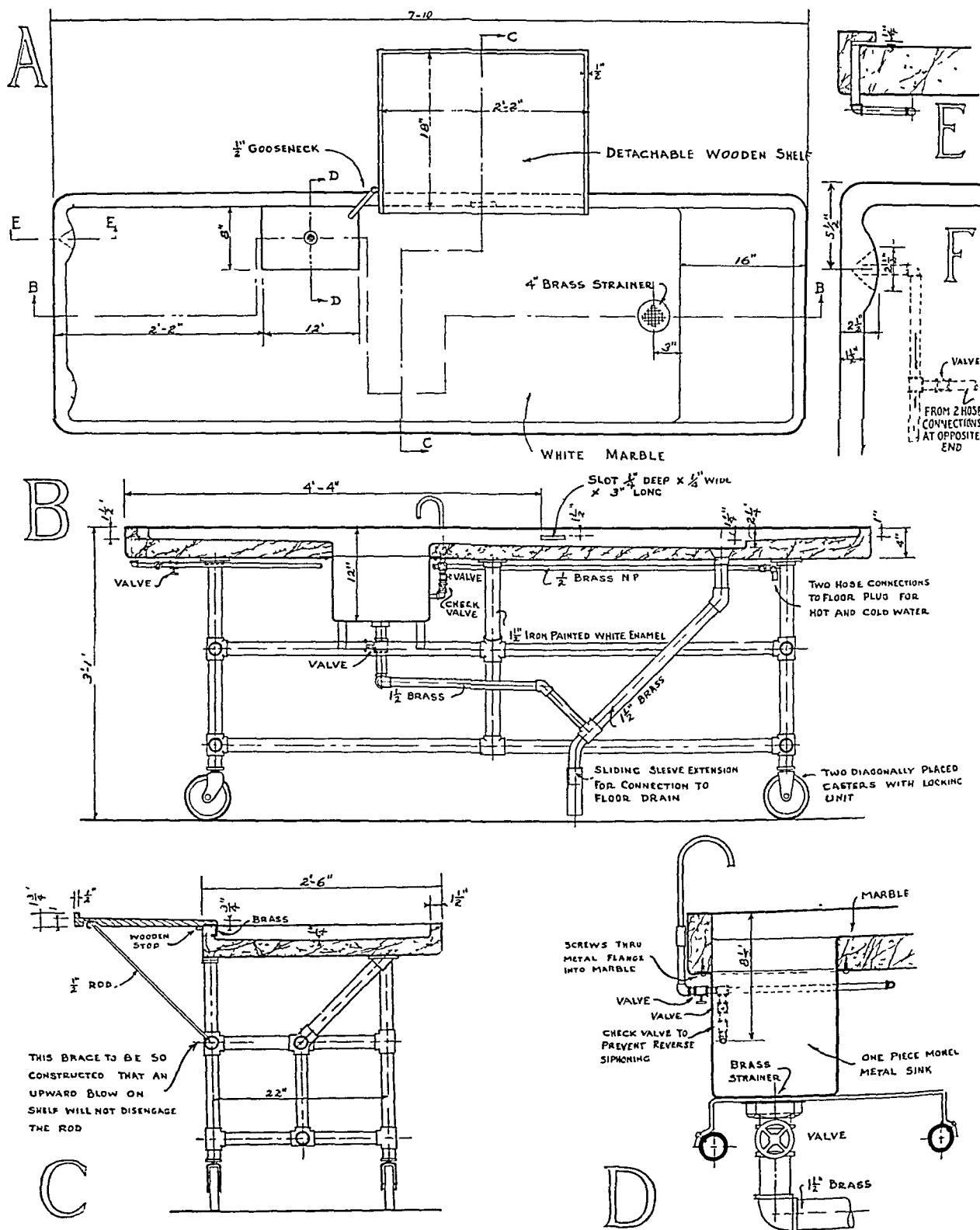


Fig. 3.—Detail drawings of autopsy table. It will be noted that there are certain variations between the specifications, the photographs and the drawings; the drawings have been made to show the more recent changes and improvements that have been made since the original table, shown in the photographs, was constructed. A is the top view plan. The lines B-B, C-C, D-D and E-E shown in this view indicate the sections the plans of which are shown in B, C, D and E. F shows the plumbing detail at E.

junction of the two. This shelf is also graded, the fall from the foot to its edge, just below the main drain grid, being  $\frac{1}{4}$  inch (0.61 cm.). No grade from the sides of this shelf to the midline is necessary.

5. Twenty-four and a half inches (62.23 cm.) from the head of the table (inside measurement), a 12 by 8 inch (30.48 by 20.32 cm.) block of the table top is cut out, the outer margin of this opening being formed by the  $1\frac{1}{2}$  inch (3.77 cm.) margin of the table (fig. 3 D).

6. At the head of the table, the top is bored with openings for two water inlet pipes, the details of which are shown in figure 3 E and F, and the locations of which are seen in the top view (fig. 3 A). The width of the rim at the location of these openings is increased to  $2\frac{1}{2}$  inches (6.27 cm.) for reinforcement. Water flows on the table from these pipes through a fan-shaped opening in the marble rim. See figure 3 F.

7. At the foot of the main part of the table in the midline is placed an opening through which passes a  $1\frac{3}{4}$  inch (4.33 cm.) drain pipe. Over this opening and countersunk, flush with the surface, is placed a brass strainer of  $\frac{1}{4}$  inch (0.61 cm.) mesh and of 4 inch (10.16 cm.) diameter.

8. Into the left side of the table top—that is, the inner surface of the left margin—52 inches (132.1 cm.) in over-all measurement from the head of the table, is carved a rectangular groove 3 by  $\frac{1}{4}$  by  $\frac{1}{4}$  inches (7.6 by 0.61 by 0.61 cm.), details of which are shown in figure 3 B.

9. The table top is attached securely to the upright posts of a cast-iron pipe frame, on which it is mounted by screws that pass through the capitals and into the under-surface of the marble. The openings for these screws should not pass through the slab.

10. On the under-surface of the table exactly in the middle and extending from head to foot is attached a  $\frac{3}{4}$  inch (1.83 cm.) water-pipe by means of brackets attached to the marble by screws. At the head end, 3 inches (7.6 cm.) from the edge, the water-pipe divides forming a "T" to supply water to the two outlets near the corners of the table (fig. 3 F). The water-pipe is connected to two supply lines, one for hot and one for cold water (located in a floor plate), by means of rubber hose. Openings in the under-surface of the marble in pairs to accommodate screws for the attachment of this water-pipe are located at intervals of 24 inches (60.96 cm.). These openings should not go through the slab.

On the left side of the table, 39 inches (98.50 cm.) from the head (over-all measurement) is located a pair of openings into the outer surface of the margin to accommodate two screws for the attachment of a bracket to secure a goose-neck water outlet, as shown in figure 3 D.

11. About the margin of the cut-out in the table top on the under-surface of the table there is a rectangular groove  $\frac{1}{4}$  inch (0.61 cm.) deep and 1 inch (2.5 cm.) wide, into which fit the flanges of a sink which is located beneath the table and attached by means of screws passing through the flanges and into the slab from beneath (fig. 3 D). Screw slots are made in the marble for this attachment, two pairs on each side and one pair at each end.

12. All corners throughout are rounded.

*Base of the Table.*—1. The table top is mounted on an iron pipe frame, all surfaces of which are covered with white enamel paint. The measurements and general plan of construction are shown in figure 3 B and C.

2. It will be noted that the carriage is not the same size as the table top. At the ends, the mount is set back 10 inches (25.4 cm.), and on the right side the carriage posts are set back 6 inches (15.24 cm.). On the left side, however, the edge of the top and that of the mount are coincident.

3. All of the various members of the frame meet at right angles, except the center upright post, which is set at an angle of about 135 degrees. This is arranged to give unobstructed working room beneath the table on this side and to make easily accessible all water connections, etc. See figure 3 *B* and *C*.

4. The four corner posts of the frame are mounted on casters, which should be ball-bearing or roller-bearing and rubber-tired, and two of which, diagonally located, are capable of being locked.

5. The table top is securely fastened to the frame mount by screws that pass through capitals surmounting the six points of support and into the under surface of the marble slab.

*Plumbing.*—1. Two drain pipes are required, the main one leading from the grid at the foot of the table and the other leading from the bottom and center of the small sink, as shown in figure 3 *B*. These pipes are of brass and should join, the common opening being 2 inches (5 cm.) from the floor and 42 inches (106.68 cm.) from the inner edge of the foot end of the frame support. Both of these drains are fitted with proper joints, so that they can be easily detached for cleansing. The drain from the small sink is guarded by a valve, with the stem pointing to the head of the table about 4 inches (10.16 cm.) below the base of the sink.

2. Water is supplied to the table at four points by branches from a main lead which begins at the foot of the table in the midline. From this point it passes toward the head, giving off one branch that supplies the goose-neck outlet, 39 inches (98.50 cm.) from the head (over-all measurement), and two branches, opposite to each other, 3 inches (7.6 cm.) from the head. The latter two branches penetrate the marble top and end in a splash plate arrangement as shown in figure 3, *E* and *F*.

The fourth point of supply to the table is from a branch of the goose-neck lead, which enters the bottom of the small sink or, if more easily accomplished, from the side of the sink 4 inches (10.16 cm.) below the table top. Four valves should be placed in this system, all under the table: one to control the inlet to the goose-neck, one to control the supply to the small sink, one to control the two outlets at the head of the table and the fourth, a check valve, to prevent reverse flow through the lead supplying the side or bottom of the sink.

All pipes are of nickel-plated brass. The pipes are secured to the under-surface of the table top by brackets and screws.

The connections of the main line of water supply with the hot and cold water lines in the floor are made of rubber hose.

3. For the location, measurements, etc., of the small sink see figure 3. The sink should be made of heavy monel metal. It is held in place by screws that pass through flanges into the marble top (fig. 3 *D*), and is supported on iron bands attached to the upper longitudinal members of the frame support. The connections here must be waterproof. The drain opening is guarded by brass strainers of  $\frac{1}{4}$  inch (0.61 cm.) mesh.

*Detachable Side Shelf.*—The shelf is made of wood and enameled in white (figs. 2 and 3 *C*). The shelf and its attachments are planned so that it cannot

be easily upset by striking against it from below, while it is at the same time detachable. The L-shaped piece, the horizontal part of which fits into the groove in the table margin, and the  $\frac{1}{2}$  inch (1.27 cm.) rods that support the outer edge of the shelf should be of brass.

The  $\frac{1}{2}$  inch (1.27 cm.) square strip attached to the under-surface of the shelf to serve as a stop is of wood and extends throughout the whole width of the shelf.

Cost of marble top (1930).....	\$230.00
(Bought of Hilgartner Marble Company, Baltimore)	
Cost of monel metal sink.....	62.00
(Bought of Lawrence Ellerbrock Company, Baltimore)	
Cost of cast-iron pipe frame support and necessary plumbing (Made in University's own shop).....	37.22
Casters .....	16.00
Total cost .....	\$345.22

## TRANSLUCENT PROJECTION SCREENS

WILEY D. FORBUS, M.D., DURHAM, N. C.

Any one who has listened to the multitude of just complaints from colleagues about the unsatisfactory demonstration of microscopic sections and lantern slides, to say nothing of the totally inadequate methods of demonstration of gross pathologic material, and who himself is conscious of the impossibility of satisfactorily demonstrating fine microscopic or gross details in pathologic conferences is conscience-stricken to the point of attempting to do something about it. Like others before me I have tried to solve this problem.

Realizing that one of the great difficulties, if not the greatest, is that large groups of people cannot get close enough to the projection screen to see details of microscopic projection, I have attempted to solve this problem by placing the screen in the midst of the group. Such an arrangement necessitates, of course, a very compact seating arrangement the limit of which is quickly reached. In order to overcome this, the obvious next step was to devise a plan by means of which both sides of the screen might be useful, thus doubling the number of people who might be seated in close range of the screen.

In order to accomplish the desired end, a number of materials possessing varying degrees of translucency were selected. By experiment it was found that an ordinary linen bed sheet, unlaundered, when stretched so that wrinkles disappear, possesses sufficient translucency to permit a very satisfactory image of a projected microscopic or

lantern slide field to be seen from the rear as well as from the front side. The woven structure of the material interferes in no way with the image, and the illumination of the back side is adequate to reveal the minutest microscopic detail. However, the source of illumination must be the arc-light. In my experience it is not profitable to attempt any microprojection without arc-light illumination. For ordinary lantern slides, either photographic or microscopic section slides, carbon filament lamps are quite satisfactory. The image is excellent on both sides of the screen.

A frame 6 feet (72 cm.) square was made from galvanized iron pipe three-fourths inch (1.81 cm.) in diameter. A piece of sheeting of the quality mentioned was selected and a square cut 6 inches (15.24 cm.) short of the frame in each dimension. Allowance was made for a 2 inch (5 cm.) hem along all four sides. This hem was securely stitched. At intervals of 1 inch (2.5 cm.) all along the



Fig. 1.—Photographs of a lantern slide projected on the translucent screen: *A*, that made from the side of the screen occupied by the projector; *B*, that made from the rear of the screen. The distance from the camera to the screen was 20 feet (6.09 meters); the time of exposure, one minute; the camera lens, Zeiss 1124404, Tessar 1:4.5, F-18 cm.; the aperture, 16; the film, par-speed; the printing time, five seconds; the developing time (print), from one to one and one-quarter minutes; the printing paper, Azo 3; the projector, Bausch and Lomb 113794, of from 110 to 125 volts, 500 watts.

four sides, round holes were punched and brass eyelets stamped in. The screen was then suspended in the frame by a continuous running cotton cord passing through the eyelets and around the iron pipe. By proper adjustment of tension it was possible to secure a perfectly flat, unwrinkled surface.

In order to eliminate the disadvantage of too great a distance between the observers and the screen, the translucent screen was suspended from the ceiling by hooks and eyes fastened to one side, the opposite side being left free. When the screen is not in use a counter weight is hooked to the free side and the screen thus

drawn up to the ceiling, leaving the room free for other purposes. The seating arrangement was so planned that the screen could be located in the midst of the group.

This technic of demonstration has been used for large clinical-pathologic conferences with great comfort and satisfaction even when the most delicate micro-projection was necessary, and when there were as many as 150 in attendance. This technic also has the added advantage of making possible the use of the autopsy room for conferences.

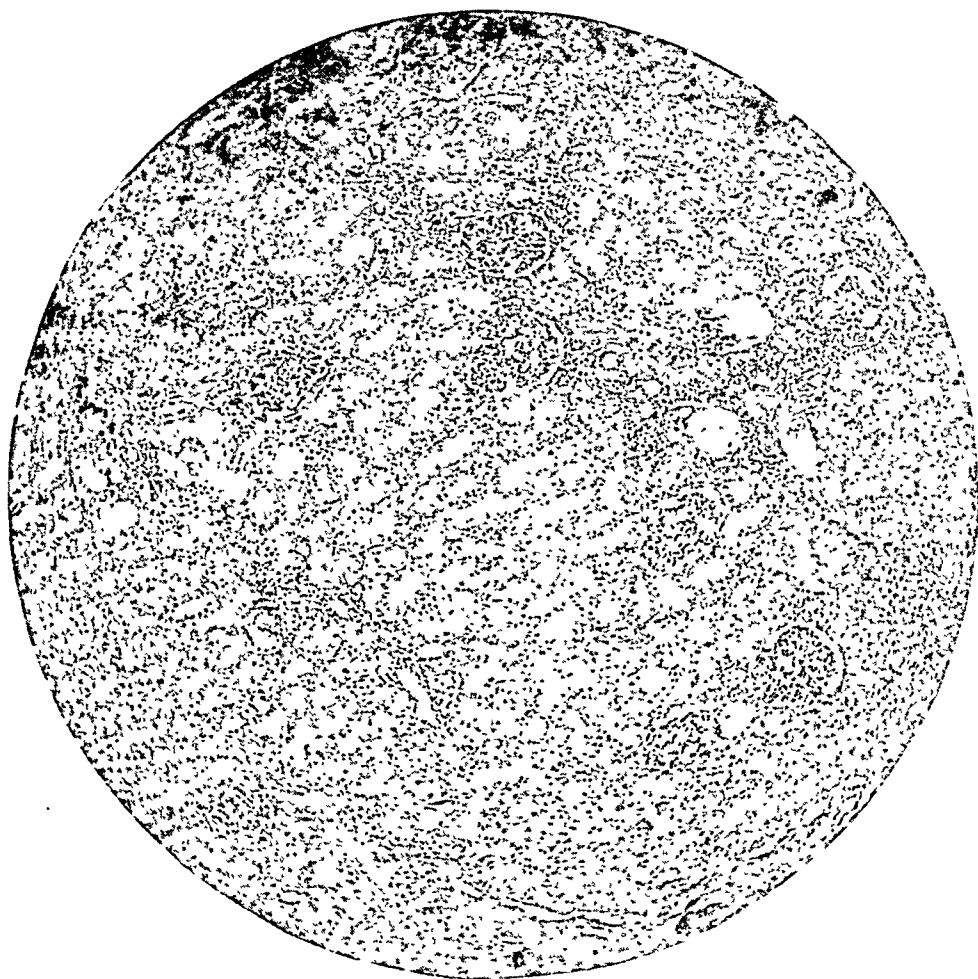


Fig. 2.—Photomicrograph of a section of kidney projected on the translucent screen constructed of sulphite paper and glass. The photograph was made from the back side of the screen. Photographs made from the front of the screen under identical conditions prove to be slightly overexposed, indicating a minor loss of illumination when the image is viewed from behind the screen. The camera lens used was Zeiss 1124404, Tessar 1:4.5, F-18, the aperture, 16; the plate, Wratten M (panchromatic); the filter, Wratten G; the exposure, forty-five seconds; the developing time, two minutes; the printing time, fifteen seconds; the paper, Azo 1; the microprojector, Bausch and Lomb 1891, with a 4.5 amperes mechanical feed arc lamp; the distance between the camera and the screen, 4 feet (122 cm.).



The efficiency of the translucent screen may be judged by comparison of the photographs shown in figure 1 *A* and *B*, which were made from before and behind the screen, all other factors in the making of the photographs of the images being kept constant in both the originals and the reproductions by the printer.

For small staff conferences of not more than from 15 to 20 people and for microscopic demonstrations, especially for small groups of students, a translucent screen of another type is necessary, since the weave of the cloth interferes somewhat with microscopic detail when the projected image is viewed at very close range. In the attempt to solve this problem I originally employed a simple piece of clear window glass, one surface of which was covered by flat white paint. Such a screen has been in use for a number of years in the Department of Pathology of the Johns Hopkins University School of Medicine, the method having been introduced, as far as I am aware, by Dr. William G. MacCallum. The difficulties experienced in obtaining a uniform distribution of the paint, which makes the chances of obtaining satisfactory and uniform illumination uncertain, suggested that a different material might be used to better advantage for covering the glass. A screen constructed in the following manner is highly satisfactory.

A square of clear window glass is first cleansed thoroughly to free the surface from all oily film and foreign particles. One surface of the glass is then covered with a relatively thick film of gum acacia dissolved in water. The solution should be quite concentrated and of a mucinous consistency. A sheet of sulphite paper (19½ pounds [8.8 Kg.] per ream) without watermark, obtainable from almost any printing shop, is first rolled loosely to avoid wrinkling and allowed to soak in water until the fiber is thoroughly wet. This roll is then placed on the gummed surface of the glass plate and unrolled. All wrinkles and creases should be worked out with the hands, leaving the paper surface flat. The paper is further flattened and the excess gum pressed out by rolling the surface with an ordinary heavy rubber double-roller such as is used in mounting photographs. Care must be exercised to prevent the separation of the fibers of the paper, since the sheet has been reduced almost to pulp by the soaking. The screen thus prepared is allowed to dry slowly and thoroughly at room temperature. Should there be some detachment at the margins of the screen, the paper can be refixed by first soaking the loose paper and applying a new film of gum. Once fixed and dried in position, the paper covering will not become detached. One should be careful to prevent any of the gum from coming in contact with the exposed surface of the paper, since this will produce a yellow stain on the paper when drying is complete.

A screen thus made may be mounted permanently in a wood frame stand. Microscopic sections can be projected on this screen at sufficiently short distances to make the finest detail of the cells clearly visible from both sides of the screen. The carbon arc must, of course, be used for illumination. A working distance between the projector and the screen of approximately 5 feet (152.4 cm.) will afford excellent projection of detail and at the same time make possible a viewing at close range by observers on both sides of the screen. The paper side of the screen should face the projector to prevent annoying reflection of the light. There will, of course, be no reflection to disturb the observers viewing the image from

the rear of the screen. The effectiveness of the screen for observation from behind is illustrated by figure 2, a photograph of a microscopic section projected on the front of the screen and photographed from behind.

These two types of screens which have been found satisfactory for every purpose have the two great advantages of permanency and cheapness. There are doubtless many materials that would serve as well as those that have been used. The selections described were made after experimental comparisons of numerous varieties of paper and cloth.

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## ROMANOWSKY STAINING OF TISSUES WITH BUFFERED SOLUTIONS

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Romanowsky staining of tissue sections has been generally unsatisfactory for routine use on account of the necessity of individual regressive differentiation of each section with alcohol, colophonium alcohol or dilute acetic acid to the desired point. It is often necessary to control this differentiation under the microscope.

Late in 1931 we had the fortunate experience of having a batch of sections stained by the MacNeall-French tetrachrome Giemsa modification come through beautifully stained without requiring differentiation. This accident was found to be due to an unusually acid sample of distilled water. This experience led us to try the effect of various buffer mixtures on stains of the eosin-polychrome methylene blue class, and the following procedure has been evolved.

Paraffin sections of formaldehyde-fixed or Orth-fixed material are brought into distilled water in the usual way and treated for two hours in a stain prepared as follows:

Eosinate of polychrome methylene blue<sup>1</sup> prepared by the Balch silver oxide method and dissolved, 1 Gm. in 25 cc. anhydrous glycerin and 75 cc. chemically pure methyl alcohol..... 2 cc.  
Phosphate buffer of  $p_H$  5.3 (M/15  $Na_2HPO_4$ , 26 cc.; M  $NaH_2PO_4$ , 65 cc.; distilled water, 909 cc.)..... 50 cc.

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From the National Institute of Health.

1. Ten grams of eosin water-soluble; 9 Gm. methylene blue, U. S. P. Medicinal, 88 per cent dye content.

They are then rinsed in distilled water, dehydrated with acetone (U. S. P.), cleared in xylene, mounted in liquid petrolatum (heavy, U. S. P.) and sealed with a pyroxylin cement.

Staining for shorter periods than two hours with the same or more concentrated or heated solutions has not given as satisfactory differential staining. Staining over night in a solution containing 1 cc. of stock stain to 50 cc. of buffer is very satisfactory and possibly slightly more differential than the two hour period, but the greater time requirement is an objection.

When it is especially desired to bring out such tissue elements as red corpuscles and eosinophil leukocytes, a buffer of  $p_H$  4.8 may be employed, but nuclear staining is not as satisfactory as at  $p_H$  5.3. On the other hand, a buffer of  $p_H$  5.6 stains nuclei and bacteria more deeply, but suppresses eosinophils and red corpuscles. Mast cells are satisfactorily demonstrated at from  $p_H$  5.3 to 4.8 and are more conspicuous at the more acid level. Mucin is better shown with less acid buffers.

With a  $p_H$  5.3 phosphate buffer, red corpuscles are orange-red; eosinophil granules, red; nuclei and tigroid granules, blue; muscle and connective tissue, pink; glia and nerve fibrils, faint pink; pancreatic secretion granules, red; liver cell cytoplasm, light blue; necrotic cells, pink, and bacteria and rickettsiae, blue. The method has proved particularly satisfactory for the study of tissues of the central nervous system.

# General Review

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## THE SCIENTIFIC BASIS OF BIOPSY IN TUMORS

C. ALEXANDER HELLWIG, M.D.

WICHITA, KAN.

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- I. Cytologic and Histologic Criteria of Malignancy
- II. Precancerous Lesions
- III. Histology and Prognosis of Malignant Tumors
- IV. Histology and Radiosensitivity of Malignant Tumors

#### I. CYTOLOGIC AND HISTOLOGIC CRITERIA OF MALIGNANCY

The microscopic diagnosis of malignancy does not seem to rest on a broad scientific foundation if Reimann's statement is true that pathologic interpretation of tissue is composed of about 90 per cent art and 10 per cent science. Fischer-Wasels emphasized that empiricism plays the principal rôle in diagnosis by biopsy.

Virchow's view that it is impossible to recognize a tumor cell under the microscope still holds today, in spite of the perfection of optical lenses and staining methods (Dietrich, Borst, Lubarsch, Sternberg, Maresch, Hertzler). By some investigators, nuclear behavior, size and number of nucleoli, loss of chromosomes, unequal nuclear division, hypertrophy of chromatin and changes of the microcentrum, of the Golgi apparatus and of Altmann's granules are thought to present criteria by which one may recognize malignant cells, but most students of the cancer problem agree that all these structures do not hold a universal key for the practical diagnosis of tumors (Da Fano, Leidenius, Horning and Richardson, Evans and Swezy, Heiberg and Kemp). Only Lipschütz believed recently that he had demonstrated a well characterized architecture of the malignant cell. By using Giemsa stain, he found in animal and human cancer cells a basophil paranuclear mass and a distinct archoplasm, which he called plastin reaction, and which he regarded as specific for blastoma. His observations were confirmed by Hirschfeld and Klee-Rawidowicz in rat sarcoma, but these structures occurred, at least in animal tumors and tissue cultures, in only a small proportion of the cells.

The cultivation of tumor cells *in vitro* has not yet given as important results from the morphologic point of view as might have been anticipated. Castrén described variations in the size and shape of cultivated tumor cells, in the chromatin content of the nucleus, in the dimensions of the nucleoli, in the cytoplasmic structure and in the position of the centrosomes that were not present in resting cells, but Hirschfeld and Klee-Rawidowicz were unable to find any specific differences in morphology between normal rat tissue and rat sarcoma when observed *in vitro*. They observed, however, distinct differences in the karyokinesis of the tumor cells as compared with normal cells. In the tumor cells there were subdiploid to tetraploid and polyploid chromosomes, incomplete equatorial plates and pluripolar, asymmetric spindles. Their observations are not in accord with those of Goldschmidt and A. Fischer, who saw only two instances of tripolar cell division among hundreds of mitoses in tissue cultures of mouse carcinoma; the number of chromosomes in their cancer cells appeared to be as a rule reduced, but normal counts were not infrequent, and in some very large tumor cells their number was doubled. Comparing the percentage of mitoses in cultures of normal fibroblasts and carcinomatous tissue, A. Fischer and Parker found the incidence of dividing cells in malignant tissue to exceed greatly that in normal tissue, in spite of the fact that the rate of growth of normal tissue may exceed *in vitro* that of carcinomatous tissue by 700 per cent.

According to M. R. Lewis and Lockwood, the fibroblasts of the Walker rat sarcoma contain twice the normal number of chromosomes, but the cells of mammalian carcinoma thus far cultivated apparently show no specific morphologic characteristics that would differentiate them from normal epithelium.

A. Fischer's physiologic studies on tumor cultures have thrown much light on the nature of malignant cells, but are at present only of theoretical interest. He reported in 1928 the following phenomena that he thought were characteristic for the malignant cells: (1) Under culture conditions, optimal for normal tissue, mouse carcinoma grows much more slowly than normal tissue; (2) carcinoma cells continue to proliferate in pure serum, while normal cells do not; (3) carcinoma cells grow well also in inactivated embryonal tissue extract, which is unsuitable for normal cells and, (4) carcinoma cells overgrow all kinds of homologous tissue cells. But his conclusions in the latest edition of his book on tissue culture (1930) were less optimistic, and also he had to admit that this modern method does not reveal specific, but only quantitative, differences in growth between the normal and the malignant cell.

The metabolic investigations of Warburg and his co-workers hold at present the foremost interest in the field of cancer research, but have

failed so far to lead to any practical results. In Warburg's own opinion, they confirm the standpoint of the histologists that there are no principal, but only gradual, differences between benign and malignant tumors. Attempts to diagnose human tumors on the basis of Warburg's observations have met with failure (Lauros).

Roffo's claims that the determination of the hydrogen ion concentration in tissue cultures permits, or excludes, a diagnosis of cancer were refuted by Bottin.

Human cancer cells apparently are not as well adapted to cultivation as those of animal tumors. The few workers who have undertaken this difficult task (Kikuchi, Castrén, Lemmel and Löwenstädt) have obtained only very scanty emigration of single cells in vitro, the nature of which was not even always fully established. Lauche reported that his culture of cancer of the human breast was the oldest one on record, since it showed mitotic figures in tumor cells three weeks after explantation. His observation in a mixed culture of human cancer and embryonal heart tissue, where the normal heart cells had overgrown the tumor tissue, makes one extremely cautious in drawing definite conclusions on human pathologic changes from the present results of tissue culture. Kredel seems to be the only one to assert that the method of tissue culture is a practical means for studying the cellular components of human tumors, at least of tumors of the brain. He obtained in 62 per cent of 37 tumors of the brain a satisfactory growth in vitro and observed a characteristic appearance and behavior of the outwandering cells for each type of tumor. Much less successful were cultures of human meningiomas in the hands of Buckley and Eisenhardt; in but 1 of 22 tumors was an outgrowth of cells obtained that could be considered as tumor tissue.

The study of experimental cancer has also failed to establish reliable cytologic criteria of malignancy. Yamagiwa and Itchikawa, who were the first to succeed in producing tar carcinoma in rabbits, pointed even then to the difficulty of distinguishing very young tar carcinoma from benign folliculo-epithelioma. As a feature representing the initial step in malignant transformation, they recognized that some or all of the epithelial cells assume a fainter stain with hematoxylin than does normal epithelium or benign papilloma. Fukuda stained sections of early tar cancer with Bielschowsky's method. Whereas in normal epithelium only the deeper site of the basal cells is clear, in tar carcinoma the interpapillary epithelial processes became clearer with the progress of epithelial growth, until finally whole cell layers failed to take up the silver stain.

Deelman and van Erp, studying the epidermoid changes due to application of tar in mice, arrived at the conclusion that cytologic criteria do not at present permit a differentiation between regenerating,

precancerous and cancer cells. Also Jaffé and Eliassow emphasized that microscopic methods fail completely in diagnosing the beginning stage of experimental tar cancer. Of their 9 rabbits in which histologic examination of excised tissue from a new growth revealed definite cancer, only 4 developed a progressive malignant tumor, while in 5 the lesion proved to be harmless.

Schuster examined skin lesions of white mice after different periods of application of tar for the purpose of studying the arrangement of chromosomes in the development of carcinoma. There were typical mitoses in the hyperplastic, papillomatous and precancerous stages, while in developed tar carcinoma he found only slightly atypical figures in the well differentiated carcinoma cells and marked atypical arrangement of the chromosomes in the immature tumor cells. He concluded that the origin of cancer is not due to a primary change in the chromosome constitution—as Boveri and von Hanseemann hypothesized—but that irregular mitoses are secondary changes, due to excessive cell proliferation.

A reliable cytologic diagnosis of cancer would be of the utmost practical importance in the examination of pleural and ascitic fluid. The most thorough investigation of this particular field was undertaken by Quensel, comparing his findings in pleural and ascitic fluids with anatomic observations at operation and autopsy. He employed supravital staining of moist preparations with methylene blue (methylthionine chloride, U. S. P.)-cadmium and sudan-cadmium, and stated that he was able to recognize malignant cells in most of his carcinomatous exudates. Quensel, however, pointed out that there is no general cytologic formula that can be applied in all cases, because the number and form of the tumor cells vary greatly. The cytologic picture depends on the nature of the primary tumor and on that of the malignant process in the serous cavity. In scirrhus carcinoma, the number of tumor cells in the exudate is small, or the cells may be absent altogether, while in medullary cancer a larger number of cells is usually found. In carcinomatous exudate, the malignant cells may occur singly or in groups. Endothelial cells are found more commonly in thin sheets, while tumor cells are arranged in different levels. The tumor cells are frequently larger and often stain more deeply than the endothelial cells, and their nuclei are often unusually large. As a most important characteristic of malignant cells, Quensel found large size, increased number and irregular form of the nucleoli.

Henke and Dietrich doubted, in spite of Quensel's valuable contributions, whether a reliable diagnosis can be made from single cells in body fluids, urine, stomach contents or feces except when small fragments of tissue are obtained.

Foord, Youngberg and Wetmore found in all serous fluids from carcinoma of the serous cavities masses of large cells, irregular in size and shape, often vacuolated and sometimes with mitotic figures. The presence of these cells in the exudate was regarded by these authors as highly suggestive of a malignant condition, but they held that a definite diagnosis is more safely made on the embedded sediment, in which fragments of tumor tissue can often be found. Confusion results in examination of smears when degenerated forms of large nucleated cells, either serosal desquamations or cells of the macrophage type, are seen. Arnstein and Huppert reported cytologic observations in 115 pleural effusions. They pointed out that effusions from various sources—hydrothorax, tumors and inflammatory conditions—cannot be safely differentiated by morphologic examination. According to Koeniger, Stadelmann and O. Fraenkel, peculiar cells of signet ring shape are so frequently present in pleural and peritoneal exudates of cancers as to be characteristic of cancer. But Henke and Andres denied their diagnostic significance, since they found these cells in cases that proved not malignant at necropsy and, on the other hand, they were absent in cases of certain malignancy.

Few authors today rely on the cytologic diagnosis in examining surgical specimens. The rapid method of Dudgeon and Patrick consists in scraping off cell smears from the cut surface of the doubtful tumor with a sharp knife, fixing the smear for from two to ten minutes in Schaudinn's fluid and, after washing in alcohol and distilled water, staining the slide with hemalum and eosin. This method failed in only 9 of their 200 cases. The variation in the nuclear size is utilized by Dengler as a criterion of malignancy. A rice seed-sized piece of tissue removed from the suspicious part of the biopsy specimen is dissected with the help of two needles in physiologic solution of sodium chloride. After addition of a drop of 1 per cent acetic acid, this preparation is examined with high magnification, and the nuclei of the tumor cells are counted according to their size. The smallest nuclei present are designated as 1, the nuclei twice the size as 2, and so on up to 7. The result is expressed as the quotient of the number of different sizes counted divided by the total number of nuclei counted. If 50 nuclei are counted, the quotient for carcinoma is always above 0.1; usually it is 0.14. Dengler applied his method successfully in 100 fresh operative specimens.

Babes recommended that in suspected cancer of the uterine cervix cell smears be examined in place of diagnostic specimens, the excision of which, he regarded as too dangerous. The suspicious lesion is wiped with gauze, and the material is taken with a platinum loop, fixed with alcohol and stained on the slide. A diagnosis of carcinoma is made from the atypical character of the epithelial cells. In the hands of Babes, this method gave 18 positive results in 20 cases that proved to be carcino-



matous at operation. Viana, employing the same technic, confirmed, by paraffin sections, the diagnosis from the smears in all of his cases of carcinoma.

In Schiller's opinion, cell smears alone are not sufficient for a reliable diagnosis of cervical carcinoma. But by removing the squamous epithelium in its entirety from the substratum, he was able to diagnose correctly 242 lesions of the cervix uteri. After the cervix has been painted with compound solution of iodine, normal epithelium becomes dark brown within a few seconds, while a pathologic surface stays unstained. Iodine negativity attracts attention to suspicious areas in which the epithelium is pathologically changed, but not necessarily carcinomatous. The nature of the pathologic process can be determined by histologic examination of the curetted epithelium. In sections, carcinoma is diagnosed, according to Schiller, from the characteristic atypical and polymorphous appearance of the cells. Deeply penetrating growth clinches the diagnosis, and can be shown by serial sections, but is not absolutely necessary to establish the diagnosis of carcinoma. Additional histologic characteristics of malignancy are sudden sharp transition of normal epithelium into carcinoma, disappearance of glycogen in the section stained by the method of Best and a sudden increase of oxygenophilia with the potassium permanganate stain of Unna. According to R. Meyer, the glycogen reaction is unreliable for the diagnosis of malignancy and according to Frankl the whole method of Schiller is still in the experimental stage.

While these cytologic methods relied more or less on the arrangement and the polymorphous character of many tumor cells in a given case. MacCarty claimed that he could make the diagnosis of malignancy from a single cell, and that in his experience the "single cell diagnosis" had never failed. According to him, there is no specific staining reaction for the cancer cell, but there are certain physical criteria by which it may be recognized in most, if not all, instances. MacCarty found the mitoses in malignant cells sometimes multipolar, but never asymmetric or irregular. The nucleolar-nuclear ratio in cancer cells is approximately 1:23, while in regenerative cells it is 1:50 or more. The regenerative cell is more delicately constructed, and its nuclear granules are usually finer. Malignant cells actually exist, according to MacCarty's belief, before the invasion of tissue—for instance, within milk ducts of the mammary gland—and they are microscopically indistinguishable from cells in lesions which all pathologists call cancer. Still MacCarty did not call these cells carcinomatous when inside of milk ducts, and he did not advise, in these cases, a radical operation, since none of his patients in whom these atypical cells had been found had died of cancer. With this conception, the "malignant cell" of MacCarty loses all practical significance. I am unable to detect anything in MacCarty's description

which has not been known since Lebert's and Hannover's unfortunate attempts in the middle of the last century to characterize morphologically the "specific cancer cell." Neither in his numerous papers on this subject, nor by his drawings and photomicrographs, did MacCarty explain how his single cell diagnosis is achieved, and he did not advance evidence in support of his assertion that the specific cancer cell is the key to diagnosis and prognosis.

Only in the special field of tumors of the brain does the cytologic diagnosis seem to be feasible. Eisenhardt and Cushing adopted the supravital technic as the most favored routine method of diagnosing tumors of the central nervous system, this being of particular value in the cytologic differentiation of the various types of glioma. A minute portion of the fresh specimen is separated with dissecting instruments and placed on a glass slide. A drop of aqueous solution of neutral red (1:10,000) is added directly to the tissue and a cover glass placed over the tissue, which is carefully spread by gentle pressure on the cover glass. This method offers an opportunity for the study of cells that have not undergone shrinkage and distortion as a result of fixation and cutting, but that are seen in their entirety while they are still living. Under oil immersion, single cells, such as astrocytes and oligodendroglia, may be beautifully defined with all their delicate processes, which are rarely seen intact in fixed histologic sections.

The finer study of cell characteristics is of great importance in the diagnosis of carcinoma of the cervix according to R. Meyer. He admitted that Schiller's new method of scraping off the surface of suspicious lesions of the cervix may often furnish sufficient material on which to arrive at a positive diagnosis, but held that it did not in every case. It will fail sometimes because cancer cells may be well differentiated and of almost typical structure, while in healing erosions immature cells of very suspicious appearance may be found. Lack of differentiation of the epithelial cells, as compared with the normal prototype, and such nuclear abnormalities as greater size and hyperchromatosis form the basis for differentiation of very early cancer and inflammatory conditions, but these cell changes must be found in all epithelial layers and over a large area of the tissue to be of decisive value. Mitoses and especially irregular forms are often missed in young stages of carcinoma. On the other hand, mitotic figures seem, in Novak's opinion, to be suggestive of malignancy. He stated that he had almost never seen them in the basal layer under benign conditions and did not recall ever having found them in the superficial layers except in malignant conditions.

The cytologic changes that are so valuable in the diagnosis of early cancer of the cervix are much less helpful in cancer of the uterine body. Benign hyperplasia may reveal thickly crowded glands in some of which

the epithelium may be in several layers with dark-staining nuclei. Mitoses may be numerous. Structures like these have more than once been wrongly diagnosed as malignant adenoma (Novak). In advanced stages of adenocarcinoma, the glandular architecture is so characteristic that a few glands in curetted material will permit a positive diagnosis (Schottländer). The carcinomatous glands are not only much longer and wider, but often labyrinthine owing to irregular papillations and complicated branching. Multiple cell layers are usually found, at least in some glands. The stroma between the glands is almost completely missing. But these changes are to be considered as evidence of malignancy only if encountered in the endometrium itself. In polypi of the uterine body, marked proliferation and irregular branching of the closely packed glands and stratification of their epithelial lining have to be regarded as benign changes as long as they are restricted to the polyps. Hintze observed 17 cases of these polyps in a twenty year period, and in none of them was malignancy observed later.

In the microscopic diagnosis of early cancer of the skin, the most valuable single factor is the assumption of younger, more vegetative qualities on the part of the hyperplastic cells involved (White and Weidman). The cytoplasm of the cancer cells has a rounded off contour; its substance is more hyaloid than normal and is different in tint from the normal.

In tumors of the bladder, also, the microscopic diagnosis rests often on atypical cell changes. In Aschner's material, 30 of 138 papillary carcinomas did not show in the biopsy specimens any evidence of invasion of the pedicle. The cytologic changes that branded these tumors as malignant were either slight and limited to a small part of the growth or marked and present throughout the papilloma. The changes on which Aschner based his diagnosis of malignancy were the presence of large cells with irregular, deeply staining nuclei, cells with atypical mitoses, pyknotic nuclei or lobulated forms, intensification of cell membranes with a tendency toward the squamous cell type, loss of palisade arrangement and loss of uniformity. Ewing stated that for a long time he had noted that the presence of a few atypical cells in a papilloma of the larynx generally signifies that the disease will recur and will kill the patient sooner or later; and that in tumors of the bladder the presence of small groups of atypical cells in an otherwise benign papilloma carries with it an increased gravity in prognosis.

In 1908, Lubarsch stated that in principle it is permitted to diagnose cancer only when one has found sure and clear criteria of destructive growth. Orth, Cornil, Borst and others regarded as the most important criterion of glandular carcinoma the perforation of the basement membrane. There is general agreement that invasion of tumor cells into the surrounding tissue and destruction of the host organ are

conclusive evidence of malignancy, even when cytologic criteria of malignancy are absent. Basing his judgment on 108 cases of malignant goiter, Graham arrived at the conclusion that it cannot be decided by cytologic characteristics whether an adenoma of the thyroid gland is benign or malignant. The only morphologic evidence of malignancy in tumors of the thyroid gland is the invasion of blood vessels.

Aschner found among his large number of tumors of the bladder a small group of infiltrating carcinomas that were characterized by a cell growth indistinguishable from that of benign papilloma. Yet the tumor invaded the pedicle, infiltrated the wall of the bladder and its vessels and eventually produced distant metastases. In these rare instances, the diagnosis could not be made, unless tissue had been removed from the infiltrated base.

Since Virchow reported several cases of metastasizing enchondromas, it is known that almost any histoid, microscopically benign tumor—myoma, lipoma, hemangioma—can produce distant metastases. R. Meyer described a sarcoma of the endometrium of typical cell structure, the malignant character of which could be recognized only from the destruction of uterine glands.

There are, on the other hand, processes with undoubted heterotopic growth, which are entirely harmless; for instance, adenomyoma of the uterus and other organs. In Ewing's and R. Meyer's treatises, there are photomicrographs of a minute benign myofibroid invading the lumen of a uterine vein. In erosion of the cervix, there may be a marked dipping in of squamous epithelium independent of the glands. The histologic changes in highly epidermoid polypi of the cervix may also be easily mistaken for cancer, especially if squamous epithelial cells have advanced deeply into the glands and have replaced the cylindric lining. The misinterpretation of this entirely benign intermingling of squamous and cylindric epithelium in cervical erosions and polyps still leads to hundreds of unnecessary hysterectomies (R. Meyer).

A common error in the diagnosis of urethral caruncle in the female results, according to Ewing, from the deep normal invaginations of the epithelial lining of this structure. On section, these invaginations may appear as displaced islands of atypical epithelium, strongly resembling epithelioma. Only the uniformity as regards size and appearance of the epithelial cells, and the fact that for the most part they are properly oriented, dispose of the suspicion of malignancy.

The human epidermis has the capacity to regenerate to such a degree as to simulate cancer morphologically (White and Weidman). In the presence of cutaneous defects known to be chronic the significance of carcinoma-like hyperplasias should be heavily discounted. Deep permeation, below the level of the sweat glands, is the only certain histologic criterion in some cases. Sweat duct epithelium readily becomes

hyperplastic in connection with suppurative and irritative processes and may be sufficiently irregular to suggest carcinomatous changes. According to Frei, the so-called carcinoma-like condyloma acuminatum of the penis is not seldom mistaken for true cancer from the microscopic structure, especially in the presence of horny pearls.

Heterotopic growth is known to be especially confusing in biopsies of laryngeal lesions. Paltauf never diagnosed a section from the larynx without knowing the clinical history of the case. He stated that after treatment with caustics the epithelium may invade irregularly the deeper structures. Especially in tuberculosis of the vocal cords, the epithelium frequently invades the underlying tissue and may even penetrate into the cartilage (Manasse). Hajek collected several cases from the literature, in which experienced pathologists had diagnosed carcinoma from biopsies, but in which the removed larynx showed only tuberculous inflammation.

Invasive epithelial growth is therefore not always identical with carcinoma, and—on the other hand—in biopsies of very early cancer destructive and heterotopic properties may be missed.

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## II. PRECANCEROUS LESIONS

No morphologic method can decide whether a noncarcinomatous atypical cell proliferation will actually develop into cancer or prove to be entirely harmless. The deciding factor when such a lesion results in a malignant tumor is apparently an individual predisposition the nature of which is completely unknown. By inoculating a saturated solution of scharlach red in the rabbit's ear, Fischer-Wasels produced atypical epidermoid growth, which completely resembled that preceding cancer, but which never attained malignant character unless the animal received the smallest doses of arsenic. The study of experimental cancer has not offered any histologic criteria for deciding whether a precancerous tar papilloma will be followed by autonomous malignant growth or remain self-limited.

Ewing's contention that in a given case one may be dealing with a condition in the process of becoming cancer may be theoretically true, but cannot be proved with present methods. The confusion is increased by using interchangeably the terms "precancer" and "beginning carcinoma." Radical pathologists like Schauenstein, Schottländer and Rubin classify as beginning cancer the same atypical tissue changes which, lacking absolute histologic criteria of early malignancy, may prove to be harmless epithelial proliferations. The terms "precancerous lesion" or "potential malignancy" have been too often used as a basis for unnecessary radical operation. There is no reason why such a case cannot be watched by a competent observer for a few weeks, when

a new exploratory excision can be made, or until more definite though still early signs develop (Frank). A long-continued follow-up by Pemberton and Smith of patients whose cervical specimens were microscopically suspicious failed to reveal a later development of carcinoma in any instance. Ewing's writings indicate that he favors the theoretical conception of a precarcinomatous stage of epithelial proliferation passing by insensible gradations from normal cells to fully developed cancer, but that in practice he regards changes without definite signs of malignancy as benign and does not permit including "precancerous lesions" in the statistics of cured cancer.

As chronic endocervicitis and laceration of the cervix, as well as hyperplasia of the endometrium and submucous myofibroids, are commonly found present with or preceding cancer, these conditions are often classed without sharp critique as precancerous etiologic factors (Villard and Montel, Ter-Gabrielian, Bailey, Lahm, Hofbauer). No statistics taking into consideration the percentage of these changes in cancerous and noncancerous women have ever been compiled, and it would seem that the frequency of these benign conditions alone disproves the theory of an etiologic relationship with cancer.

Leukoplakia of the female organs was described first by von Franqué as a precursor of carcinoma. Hinselmann regarded it in the cervix not merely as a precancerous condition, but as an early noninvasive stage of carcinoma. Both views were rejected by R. Meyer, who had often seen multiplicity of cell layers, with elongation of rete pegs and papillary overgrowth of spinous cells, sometimes with hornification, in cases that by the clinical course proved to be benign. In their study of 21 cases of vulvar carcinoma, Graves and Smith found leukoplakia 16 times and kraurosis 14 times, and they feel that there is a close relationship between these two processes and cancer. Of Taussig's 29 cases of vulvar carcinoma, 60 per cent showed the malignant changes taking place in an early hyperplastic area, and 40 per cent. in a late atrophic area. In Taussig's opinion, squamous cell carcinoma of the vulva springs from leukoplakic vulvitis in almost every instance, and the latter undergoes malignant change eventually in at least one half of the cases. Rentschler, on the other hand, in a review of 71 cases of primary vulvar carcinoma, found that in 40 per cent the patient gave a definite history of preexisting pruritus, but that only in five cases was leukoplakia coexistent with carcinoma.

Kutzmann observed 67 cases of leukoplakia of the renal pelvis, 11.9 per cent of which were combined with carcinoma. Among 3 cases of leukoplakia of the urinary bladder, von Borza saw 2 in which this organ was also affected with squamous cell carcinoma. Kretschmer believed that leukoplakia of the urinary organs is probably much more common than it is generally thought. In only 1 of his 13 cases was carcinoma

associated with leukoplakia of the bladder. By collecting from the literature the cases of leukoplakia and carcinoma of the urinary organs, Patch hoped to answer the question whether leukoplakia and cancer are as intimately related to each other as is generally accepted. Of the reported 121 cases of leukoplakia and 152 cases of squamous cell carcinoma of the urinary tract, only 13 showed coexistence of both processes.

According to Brüning, leukoplakia of the buccal mucosa is often followed by carcinoma. No unanimity of opinion, however, exists in regard to the frequency of this transformation (from 10 to 53.8 per cent). Lazarus believed that one third of all cancers of the tongue arise from leukoplakic areas. Herzen and Jenckel insisted on radical excision of all leukoplakic areas in the mouth as a prophylactic measure. Also Deelman called the leukoplakia that occurs in the upper parts of the respiratory and alimentary tracts a true precarcinomatous change. He found at the periphery of the carcinoma that develops in leukoplakia the gradual transition from hyperplastic epithelium to neoplasm and the morphologic substratum of precarcinoma in its final development. Schaer, on the other hand, doubted whether leukoplakia plays as important a rôle in the genesis of cancer as many believe. He found in 237 esophagi without cancer, obtained at autopsy mostly from men above 40 years of age, 67 per cent affected with leukoplakia. In his series there were 17 traction diverticula that not seldom showed atypical proliferation of the surface epithelium, and Schaer was of the opinion that these diverticula may predispose to the development of esophageal cancer much more than leukoplakia. Sharp, however, concluded from one observation of a case in which relatively early carcinoma was coexistent with far advanced leukoplakia of the esophagus, that the irritating factors may bring about a leukoplakic response in one area, and that the same or additional factors may cause cancer in another.

Benjamins described 3 cases of laryngeal carcinoma with continuous transitions from precancerous changes to fully developed carcinoma. He concluded from these observations that every papilloma showing hyperkeratosis should be treated as a malignant tumor. Also Tucker and Jackson regarded the evidence as conclusive that cancer of the larynx develops from nonmalignant lesions.

There is wide difference of opinion regarding the possible transformation of chronic gastric ulcer into carcinoma. While Konjetzny placed the percentage at 3 and Hauser at 6, Wilson and MacCarty reported it as occurring in 71 per cent. Klein and Demuth studied ulcers and carcinomas of the stomach with the aim of determining whether any histologic changes other than those easily recognized as definite carcinoma might be considered characteristic of malignant transformation of

an ulcer. They doubted that the histologic diagnosis can be based—as Moszkowitz believed—on deeply stained epithelial cells, no matter how atypical or heterotopic they are.

Stewart believed that an important and intimate relationship exists, both in the stomach and in the large intestine, between simple adenomatous polyp and carcinoma, but the ultimate full development of a malignant condition is in most cases confined to a single focus. Hurst found in about 0.5 per cent of his cases of gastric carcinoma diffuse gastric polyposis and in 4.5 per cent simple adenoma as a precursor of malignancy. According to Miller, Eliasow and Wright, carcinomatous polyps of the stomach are more frequent than has been generally believed; both the history and the pathologic data suggest that they usually originate in polyps of a benign nature. The authors observed 8 cases of carcinomatous polyps in a series of about 200 operations for cancer of the stomach, constituting about 4 per cent of the group.

In adenoma of the stomach and of the intestines, Bloodgood advised radical resection as a prophylactic measure. According to Lockhart-Mummery, Dukes, Rankin and Bagen and many others, rectal adenomatous polyps constitute the most dangerous predisposing factor in the development of rectal adenocarcinoma. The adenomas are usually numerous and often separated from one another by normal mucosa. The bowel may remain in this condition for many years, or at any stage of the epithelial proliferations the epithelium may abruptly assume those invasive properties recognized clinically and histologically as cancer. The incidence of malignancy in multiple polyposis is generally accepted as being from 40 to 50 per cent. But according to Bagen and Rankin, it is impossible from histologic and clinical examination to predict whether or not a given polyp will become malignant. While most of the authors did not have the opportunity actually to observe a malignant transformation in a particular polyp in any case, Schnieden and Westhues described all histologic transitional stages from harmless adenomatous hypertrophy of the mucosa to polyps with partially undifferentiated cells leading up to fully developed adenocarcinoma. Westhues classified the different histologic pictures into 3 groups. His third—which alone he calls the precarcinomatous stage—is characterized by invasion of the pedicle and perforation of the muscular layer, but there arises the question whether this third stage with definite signs of malignancy should not be called carcinoma. Westhues' classification of intestinal polyps into 3 groups is accepted by Fitzgibbon and Rankin. In the tumors belonging to their third group—the definitely precancerous lesions—the cells could not be distinguished morphologically from those seen in outright cancer. For 19 of their 24 cases of cancer they found indisputable proof that the sites of origin of the malignant growths were in polyps, and they concluded that cancer of the colon develops usually



from an earlier stage as a polyp. Fitzgibbon and Rankin regarded it, however, as an impossible task to determine how many polyps actually become cancerous.

This difficult problem was attacked by Feyrter. He examined at autopsy the large intestines of 1,110 patients over 20 years of age and found in about one third of them adenomatous proliferations in the colon. The total number of polyps found was about 1,000, but only 6 of these were carcinomatous and 4 suspicious for malignancy.

Of great interest is the case reported by Hullsiek of multiple polyposis of the colon, in which the first biopsy did not show any histologic evidence of malignancy. Two years later an adenocarcinoma was revealed by biopsy at the rectosigmoid junction. The autopsy showed, besides this carcinoma—with no suggestion of its having arisen from a polyp—no carcinomatous changes in any of the polyps that extended from the rectum up to the ileocecal valve. Observations of this kind led Stahr to the conclusion that rectal polyps like other atypical epithelial proliferations are not true precancerous stages, but can be regarded only as indicators of some kind of irritation that is capable eventually of producing cancer under circumstances not yet understood.

There is bewildering dissension about the practically important question whether chronic cystic mastitis has to be regarded as a precancerous lesion. In my opinion, this problem will never be solved by present histologic methods alone, but only by long-continued clinical observation of nonradically treated cases. The morphologic appearance of atypical epithelial proliferations within ducts and acini in chronic cystic mastitis is seldom definite enough to enable a dogmatic interpretation of its biologic state. The disagreement of different pathologists in a given case of proliferant chronic cystic mastitis demonstrates well the limitations of present histologic methods. At a pathologic conference in 1931, Bloodgood invited 50 experienced pathologists to diagnose the slides of 10 lesions of the breast that were known to be clinically benign. Not in one single instance was there general agreement regarding the significance of the microscopic picture.

The fact that carcinoma of the breast is often found associated with chronic cystic mastitis is easily explained by the high incidence of the latter. Goens found at autopsy in one third of all women over 40 years of age changes typical for chronic cystic mastitis, and Hahn saw the same condition in 11 of 48 women younger than 40 years. Still higher is the incidence in the material of R. Jaffé. He observed in 100 autopsies of women over 40 years of age chronic cystic mastitis in 93 per cent. In 65 per cent, he found marked epithelial proliferations of the milk ducts that were indistinguishable from changes called precarcinomatous by many pathologists.

Cheatle believed that 20 per cent of all cancers of the breast begin with chronic cystic mastitis. The appearance of the latter disease and its subsequent course occupy the same age decades—25 to 30 years—as tar carcinoma does in man, and the carcinoma in each appears to be the result of a continuous process which as a rule takes a definite time. In early mammary carcinoma there is, according to Cheatle, an epithelial neoplasia the cells of which are still contained within their normal boundaries, and these cells resemble precisely in morphologic appearance those that have invaded the stroma. Hyperplasia of the elastica surrounding only those terminal ducts that contain epithelial cells of malignant character is regarded as a distinct indication of the dangerous state. But Cheatle also had to admit that, aside from arousing suspicion, the matter must remain a question until the biologic state of epithelial cells can be determined with greater certainty than can be elicited from their morphologic characteristics only.

Goldzieher and Kaldor examined 47 specimens of chronic cystic mastitis. In 12, they found cancer; in 4, precancerous changes. In the latter, invasive growth could not be demonstrated, yet the morphologic appearance of the hyperplastic epithelium was suggestive of malignancy. These precarcinomatous changes were identical with proliferations that were observed in the vicinity of frankly carcinomatous foci within milk ducts. The authors could not prove, however, whether or not these precancerous changes were about to develop invasive tendency, although "it seemed highly probable."

Semb made an exhaustive anatomic study of 148 cases of chronic cystic mastitis and described microscopic changes passing by insensible gradations from simple microcystic chronic mastitis into atypical hyperplasia and finally leading up to invasive carcinomatous neoplasia. He concluded that chronic cystitic mastitis is a true precancerous lesion which eventually will result in cancer and which requires removal of the whole mammary gland. Also Klose and Sebening regarded prophylactic amputation of the breast as the only logical treatment for chronic cystic mastitis. The frequency of malignant transformation after partial resection of the diseased area of the breast is in their opinion "formidable." Benda found in most specimens of chronic cystic mastitis such atypical epithelial proliferations that he felt sure that this is a precancerous condition with grave prognosis. Ewing concluded from his vast experience that chronic cystic mastitis is a very important condition predisposing to mammary cancer. From the practical standpoint of prognosis, Ewing regarded it as safe to remove the whole breast or the whole affected segment when an incision is required for diagnosis. In case of malignancy, the correct surgical procedure can be determined during exploration. The course of the established disease

is, in Ewing's opinion, generally progressive, and most cases of chronic cystic mastitis eventually terminate in carcinoma or in surgical removal of the breast.

Clinical considerations, however, suggest that chronic cystic mastitis will often not develop into cancer and show, on the other hand, that many cancers of the breast are not preceded by a precancerous stage. Of 617 women with cancers of the breast who were operated on by Låwen, only 11 had, several years before operation, chronic mastitis as proved by biopsy, and 64 had clinical symptoms of chronic mastitis many years preceding the development of cancer. Bloodgood, from the standpoint of a clinical and anatomic study of 350 cases of chronic cystic mastitis, concluded that this condition has no etiologic relationship to carcinoma, and that women with this disease are no more in danger of acquiring cancer than women of the same age without it. No matter what the operation was in his 350 cases, there was no death from carcinoma of the breast in a period of over twenty-five years. Also the so-called diffuse adenomatous variety of chronic cystic mastitis, which Bloodgood believed up to 1906 to be associated with cancer in at least 50 per cent of the cases, is, according to his recent statements, not a true precancerous lesion, its association with cancer being only incidental. In probably 70 per cent of patients with chronic cystic mastitis coming early for medical observation, operation can be decided against by palpation alone. Of the 30 per cent in whom the breast must be explored, one half or more reveal benignancy on gross inspection and examination of frozen sections. In 40 of 43 patients with chronic cystic mastitis whom I observed, only partial resection of the diseased area was done, and not one of these returned with a malignant growth. In only 3 was a radical operation advised after biopsy—this on account of intraductal proliferation so marked and so atypical that suspicion of malignancy was aroused, although no frankly invasive epithelial growth could be demonstrated. To remove all breasts with chronic cystic mastitis would, I believe, entail an unnecessary mutilation in at least 90 per cent of the cases.

Of greatest importance in evaluating properly the finding of atypical epithelial proliferation in biopsy specimens is the work of Meeker. She dissected at autopsy normal vocal cords and their attachments from persons aged from 2 months to 80 years, with no history of laryngeal lesions. In these normal specimens, great activity of the squamous epithelium with filling in of wide surface openings of the ducts and fusion into solid masses was present. Within the deeper gland ducts, papillary thickenings of the epithelium as well as a tendency to metaplasia were common. These changes were exactly like those that are called precancerous by many pathologists. Meeker's observations certainly suggest caution in basing prognostic conclusions on more or less

atypical cell proliferations—not only in the larynx, but in any other part of the body where specimens for biopsy are obtained.

In my opinion, "precancer" as a histologic diagnosis is meaningless. In diagnosing a lesion by biopsy, either the pathologist should take the full responsibility by pronouncing the lesion malignant or benign, or—if he is unable to arrive at a definite diagnosis—he should have the courage to admit that he does not know and leave the responsibility to the clinician or to a more experienced histologist. Nothing is gained by calling a suspicious change in the tissue a precancerous lesion, a term which is a confession of ignorance.

### III. HISTOLOGY AND PROGNOSIS OF MALIGNANT TUMORS

From the beginning of the microscopic era, the tissue experts have formed from their histologic observations an opinion concerning the progress of the neoplastic disease. Von Hansemann regarded it as a law that tumors disseminated through the body show a high degree of anaplasia, while those of unusual local growth without dissemination show little anaplasia. Henke, Borst, Hertzler and Herly accepted this theory in a general way, but they called attention to the frequent exceptions. In 1915, Ewing regarded as insufficient a pathologic diagnosis simply of carcinoma or sarcoma, since some processes to which these terms are attached are self-limiting, while others are invariably fatal. He believed that the pathologist is able to interpret the clinical diagnosis from the microscopic section in Hodgkin's disease, malignant lymphosarcoma, osteogenic sarcoma and many other tumors of which the prognosis is practically always fatal. In another group, the potential malignancy of the tumor may be judged with much accuracy from its histologic structure, but its actual clinical course may be subject to wide variations, depending chiefly on age, location, size and duration.

Five years after Ewing's cautious conception of the relationship between histology and clinical prognosis of tumors, Broders aroused the interest of surgeons and pathologists by his statement that the clinical course of many cancers may be quite accurately predicted by analysis of the histologic structure alone, without taking clinical factors into consideration. Basing his work on von Hansemann's law that the more highly differentiated the cells, the lower is the malignancy of a tumor, he graded carcinomas of the lip and other regions into 4 grades according to the differentiation of the tumor cells, and presented statistics to prove that these histologic features govern the life expectancy in a given case. According to him, cancer of grade 1 shows practically no tendency to metastasize, and therefore in dealing with such neoplasms he considered it unnecessary to remove the regional lymph nodes. As, on the other hand, practically all cancers of grade 4 with metastases prove fatal, he advised the surgeon not to subject patients showing these cancers

to an operation involving the regional lymph nodes, unless these nodes are in close proximity to the primary growth. In Broders' opinion, the time is not far distant when not only physicians but also the patients and their relatives, as well as life insurance companies, will be interested in the grade of malignancy of cancer. His system of standardizing histologic prognosis has been acclaimed by many surgeons throughout the country and has already influenced surgical practice. MacCarty's recent remark that a good piece of scientific observation has been spoiled by the clinician, and that clinical interpretations have gone too far, does not seem entirely justified, since Broders himself stressed the great practical value of his method. Plaut's sound criticism (1927) and his warning that the time is not yet ripe to advocate any known method of histologic grading for practical purposes have cleared the field for more extensive study.

While Ewing, Martzloff and Greenough followed Broders' system in grouping malignant neoplasms of the various organs into 3 or 4 grades of malignancy according to the relative number of undifferentiated cells present, Hueper attempted to find a more reliable index of histologic malignancy by using 20 factors pertaining to the characteristics of the parenchyma and stroma of a tumor. He used 4 principal criteria: (1) special cellular and structural characteristics, (2) characteristics of cytoplasm, (3) characteristics of nuclei and (4) characteristics of stroma, with from 4 to 6 subdivisions in each, valued from 1 to 4. The sum gave the index of histologic malignancy. Hueper emphasized, however, that the grade of histologic malignancy is certainly not the only factor on which the type of treatment should be based and the prognosis estimated. The extent of the disease as best expressed by a definite clinical grouping, the age of the patient, heredity, the general condition of the patient, the location of the tumor and its extrinsic or intrinsic qualities of growth deserve as much consideration as the histologic grading.

In his earlier studies, MacCarty had called attention to lymphocytic infiltration, fibrosis and hyalinization of the stroma as factors of prognostic value. From his clinicopathologic observations of cancer of the stomach, breast, rectum and skin he concluded that the value of these factors for determination of the clinical course of the disease could hardly be questioned. According to Ewing, well marked inflammatory reaction signifies a pronounced capacity of the organism to limit the tumor's growth, but not that the effort will be successful. He had not, he said, found the presence of lymphocytes and fibrosis a reliable sign of the outcome of the disease.

W. Fischer's and Boehmig's histologic studies on 115 nonulcerated carcinomas throw much light on the nature of stroma reactions in malignant tumors. The quality and intensity of the inflammatory changes

differed in the various areas of the same tumor so considerably that the authors warned against drawing conclusions from biopsy of small specimens. In their series, scirrhus and solid carcinoma showed much less lymphocytic infiltration than alveolar adenocarcinoma. In metastases located in the liver inflammatory reaction was never found. It was always present in early stages of cancer, while well advanced neoplasms showed only scanty local infiltration around small cell groups with active proliferation. In colloid carcinoma, the lymphocytic infiltration was absent or very small. When the tumor grew in preformed channels, such as blood vessels, lymph vessels or milk ducts, the lymphocytic infiltration was entirely missed, but it appeared as soon as the growth perforated the walls of these structures. In the opinion of Fischer and Boehmig, the stroma reaction is to be considered as inflammatory and not as specific for carcinoma; it depends no more on the structure and rate of growth of the neoplasm than on the texture of the invaded organ. The two authors would not draw any conclusions from the character of the stroma reaction on the clinical prognosis in a given case. Fischer-Wasels observed in transplanted mouse carcinoma that the lymphocytic infiltration varies widely, depending on the structure of the host organ.

Wood in 1930 stated that no one can make a prognosis from a microscopic section except in certain well recognized groups of tumors. He regarded malignancy as a clinical phenomenon, not a morphologic one. The prognosis of a neoplasm depends on many things: on the position of the growth, its relation to blood vessels, its size in some cases and the dimensions of the tumor cells wholly independent of their rate of growth.

While Wood, Ewing and Plaut recognized the great scientific and practical value of the problem of histologic grading and encouraged further investigation in this field, which still is in its infancy. Reimann regarded it as futile to decide from a small section of a tumor what will happen to a patient with cancer. According to him, in histologic grading, one attempts, by examination of the removed specimen, to determine how it grew and then to transfer this decision as a prediction of what will happen to any possible fragment left behind by the surgeon. If one is to make a prognosis at all, the presence or the absence, and the situation, of secondary tumor deposits are of greater importance than histologic grading.

The problem of grading is complicated by the well known fact that tumors of the same histologic structure, but in different locations, may have entirely different grades of clinical malignancy. Ewing in 1915 stated that carcinoma of the tongue presents a very different problem from that of carcinoma of the skin; in the alimentary tract, carcinoma increases in clinical malignancy from the anus to the lips, and also in the urinary bladder the malignancy varies according to the location, irrespective of histologic characters.

Ewing distinguished between potential and actual malignancy of a tumor. The potential malignancy can be safely estimated from histologic signs of anaplasia, abundance and abnormalities of mitoses, loss of polarity and diffuse infiltrative growth of cells. The actual malignancy is influenced by many factors that may greatly alter the course of the disease; for instance, the position of the tumor, hemorrhage, bacterial infection. Ewing pointed to the danger of relying exclusively on histologic data in forming a clinical prognosis, especially if more significant features such as gross anatomy of the tumor, condition of the lymph nodes, age of the patient, duration of the disease and other clinical aspects are thereby neglected.

MacCarty, in his latest paper on histologic grading, which differs strikingly from his former dogmatic contributions and reflects the wholesome influence of the criticism by Plaut, Reimann and Wood, admitted that all grading of cancers should be considered as in the investigative or experimental stage, and that the immature clinical utilization of scientific observation will only discredit and impede its rightful progress. He warned against using for practical prognosis the facts which he and Broders had presented, and urged that any system of microscopic grading of cancer cannot be of accurate value without many other factors being taken into consideration. There are according to MacCarty at least 15 factors governing prognosis in cancer. The most important consideration, more important than any microscopic criterion of degree of malignancy, is whether metastases are present in lymph glands or other organs, and secondly whether the surgeon could remove the growth radically.

Warthin pointed out that all degrees of anaplasia may be found in a single neoplasm, so that one's grading of malignancy will vary in a given case according to the part of the tumor from which the piece examined is taken. The questionable value of histologic grading from biopsy of small specimens, with regard to the variation of cell characteristics and stroma reaction in different areas of a tumor, is evidenced by the study of Martzloff. In 70 cases of cervical cancer, he compared material taken for biopsy with the remainder of the removed uterus, and found that in one third of the cases studied histologic examination of the biopsy material failed to show the predominant type of cancer cells as presented in the remainder of the organ. In his opinion, material taken from cervical carcinoma for biopsy will give a more or less inaccurate impression of histologic features in regard to the grade of potential malignancy.

Stewart and Spies, on the other hand, concluded from their examination of rectal carcinoma that small biopsy specimens are sufficient for the histologic grading. Having had the opportunity in 46 cases to obtain more than a single specimen from the same tumor, they found

that changes in the histologic character do not occur sufficiently often in the course of rectal cancer to interfere seriously with the prognosis as suggested by the grade given at the time of the first biopsy. In a total of 102 repeated biopsies but 10 changes in grade were made, all except 1 of these consisting in an advance of the grade.

Von Hanseemann was of the opinion that in metastatic tumors there are as a rule increased anaplasia and more rapid growth as compared with the primary tumor. According to recent investigations, however, the preservation of the structural types in metastases seems much more common, and in a few cases the grade of histologic malignancy appears even to be lowered, an observation which it is difficult to understand from the clinical point of view. Patey and Scarff studied 163 cases of carcinoma of the breast for the purpose of comparing the histology of the primary tumor with that of the metastases. In 83 per cent, the secondary deposits in the lymph glands were similar in appearance and in histologic malignancy to the primary growth. In 16 per cent, however, the malignancy of the glandular metastases was lowered, and in only 1 case was it raised. Mills, Broders and Caylor found irreconcilable variation between the grade of the primary malignant tumor and that of the metastatic growth in 32 of 207 cases. In 17 metastases, the histologic grade of malignancy was lower than in the primary tumor, and the authors observed a tendency for some carcinomas to become "a little less malignant" when they became implanted in the lungs—a statement that must arouse still greater skepticism as to the clinical value of purely histologic grading.

There are many statistics in the literature that seem to speak in favor of the histologic grading of malignant tumors in different locations of the body. A systematic analysis, such as that made by Plaut on Broders' tables, may prove, however, that most of these statistics are not without fallacy. There is particularly one point which appears to me worthy of mention and which must have a deciding influence on these figures: the uncertainty of present histologic methods in differentiating the earliest stages of carcinoma from harmless atypical cell proliferations. If the latter are listed in the statistics as cancer of low grade, an entirely wrong conception of the clinical course of the different histologic types must be induced. Broders, for instance, included in his group I, of carcinomas of the bladder, tumors that most pathologists call benign papillomas. His group II would probably fall in Aschner's class of papilloma with malignant cell groups. Then one readily understands that his classes III and IV are really malignant ones, since they are the only tumors of the bladder that are called carcinomas by most histologists. White and Weidman reported atypical epithelial proliferations on the edge of cutaneous ulcers which were graded by noted pathologists as grade I and grade II carcinoma, but which healed



promptly after conservative treatment. The difficulty of differentiating by histologic study the first stages of carcinoma of the breast from harmless atypical proliferation in chronic cystic mastitis is well known, and accounts for the everyday experience that one pathologist will seldom agree with another on the significance of the pathologic pictures in these borderline cases.

In carcinoma of the uterus, most of the efforts to establish a close relation between structure and prognosis have been announced as comparative failures. In Frank's opinion, so many incalculable factors come into play that no prognosis can be based on the histology only. Also Schottländer and Kermauner concluded from their extensive studies that the results of grading give little encouragement so far as prognosis is concerned from the examination of diagnostic excisions or curettages. R. Meyer distinguished different grades of cervical carcinoma for clinical purposes, because the unripe types are thought to respond more readily to irradiation, but he emphasized that the histologic grading of these cancers is extremely difficult at best and depends too much on the personal interpretation of the diagnostician. In his material, the unripe forms were extremely rare, and he did not see any close relation between the grade of cell differentiation and the clinical course, except that the ripe carcinomas in early stages seemed to grow more superficially, while the unripe types had often the tendency to invade the deeper structures from the beginning. But Meyer raised the question whether the density of the inflammatory stroma is perhaps not more responsible for the different direction of growth than the maturity of the tumor cells.

Lenczewski investigated the microscopic structure in 26 cases of cervical carcinoma; 12 of the patients were alive three years after operation and 14 dead. There was no relation between histology and prognosis in his cases, and the length of survival in any case seemed to depend mainly on the patient's general condition and clinical picture before treatment.

Martzloff applied his system of histologic grading to 145 cases of epidermoid carcinoma of the cervix, distinguishing spinal, transitional and spindle cell forms. In the spinal cell group there were 53.3 per cent with five year cures. In the transitional cell group—the most common variety—the occurrence of five year cures was 43.3 per cent and 34.3, respectively, in the patients operated on by the vaginal or abdominal route. Of 17 patients with the spindle cell cancer, the least common variety, only 1 was living after five years. Plaut, who applied Martzloff's method to his material at the Woman's Hospital, New York, could not find any relation between the type of cells and the outcome of the disease, with the exception of an increased number of survivals in cases with intensive hornification and a rapidly fatal outcome in those

with very irregular pleomorphic pictures. No tumors in Plaut's material could be classified as spindle cell cancers, and furthermore the cell forms in cervical carcinoma were so manifold that they did not permit establishment of a few histologic groups. He concluded that at present the clinical features are a much better guide to prognosis than the cytologic grouping.

Neither was Taylor able to find a definite relationship between histologic structure and prognosis. He applied the original method of Martzloff to 128 cases of cervical cancer. The greatest number of surgical cures occurred in tumors of grade III. The prognosis in 60 hysterectomies for carcinoma of the uterine body depended largely on the degree of gross extension at the time of institution of therapy. Also, in ovarian carcinoma, the histologic type apparently made little difference in the prognosis.

Hueper based his method of grading on 20 different histologic criteria, and 48 known end-results among 122 carcinomas of the cervix convinced him of the usefulness of his system. In group I, with a malignancy index between 22 and 39, there was unfortunately no case with a known end-result; in group II, with a malignancy index of from 40 to 54, three year cures were obtained in 61.5 per cent; group III, with a malignancy index between 55 and 69, had cures in 28.6 per cent, and group IV, with a malignancy index between 70 and 84, had not a single cure.

In McNamara's 300 cases of cervical carcinoma, the classification according to the technic of Martzloff did not harmonize with the end-results except in a few instances. Therefore he distinguished only 2 classes, that of low and that of high malignancy. In the low malignancy class he included all cancers that tended toward keratohyaline formation with pearls, the cells resembling the normal stratified squamous epithelium of the portio vaginalis. Of patients whose cancers he classified as belonging to the highly malignant group, all were dead from the disease after a three year period, while of those whose tumors were of the low grade all were living except 3 who died from other causes. The type of treatment showed little difference as regards end-results. Since McNamara grouped his material as only 15 cases of low and 276 of high malignancy, leaving almost 95 per cent of the cases unsorted, it seems questionable whether this new system will find followers.

Warren studied the problem of histologic prognosis on autopsy material and took the power of a tumor to metastasize as an index of its degree of malignancy. His 102 cases of cervical carcinoma were divided into 3 groups on the basis of differentiation of the tumor cells, frequency of mitosis and relation of tumor parenchyma and stroma. Of epidermoid carcinomas of grade I, 57 per cent had no metastases as

revealed by autopsy: of tumors of grade II, 23 per cent were without metastases, and of those in the last group all had metastasized. Warren held that in cervical carcinoma there is a close correlation between the histologic degree of malignancy and the power to set up metastases.

In endometrial carcinoma, von Hansemann maintained that, in general, histologic classification does not give any guide for evaluation of malignancy. According to him malignant adenoma of the uterine body differs in no way clinically from adenocarcinoma. Mahle, on the other hand, reviewing 186 cases of carcinoma of the fundus, divided them into 4 groups according to the amount of tissue differentiation. In the cases of grade I, which showed the least departure from the normal, no deaths occurred, while in those of grade IV every patient died. Lindsae divided his cases of cancer of the uterine body into 2 groups, adenoma malignum and adenocarcinoma. In the first, the polarity of the epithelial cells was maintained everywhere, while in adenocarcinoma definite loss of polarity and infiltration of the stroma by solid cords of cells were observed. In his 23 cases of adenoma malignum there were 12 three year cures and only 2 patients died, while in the 30 cases of adenocarcinoma there were 4 three year cures and 21 patients died. These different results were not due to differences in treatment.

R. Meyer held that in adenomatous uterine cancer, the insensible transitions between different types—not only from case to case, but also in different areas of the same tumor—do not permit a distinction between malignant adenoma and adenocarcinoma. The texture of the stroma, the presence or the absence of inflammatory changes and other factors may alter the histologic structure of a tumor considerably without changing its growth capacity.

In 1922, Sistrunk and MacCarty published a paper based on the study of 218 breasts that had been radically removed for carcinoma. They found that the greatest combined factors controlling longevity after operation were cellular differentiation and hyalinization of the stroma. According to Flothow, who studied 222 cases of mammary cancer, hyalinization of the stroma is the most effective of the tissue reactions and constitutes a practically impassable barrier to the advance of malignant cells. Lymphocytic infiltration, which is the first reaction to appear, is regarded by him as the least important defensive factor.

Greenough, however, basing his opinion on 73 carcinomas of the breast, could not confirm the view that round cell infiltration and hyalinization of the stroma are an indication of the resistance of the individual against cancer. In his belief, round cell infiltration is called forth by the presence of degenerative products in the tissues, and hyalinization is rather a factor of age or previous condition of the mammary tissue. Greenough considered the presence of marked secretory activity with droplets of mucoid secretion as an indication of low

malignancy and its absence as that of a lesser degree of differentiation. A marked variation in size of cells and nuclei combined with hyperchromatosis and large numbers of irregular mitoses carries in his opinion a grave prognosis. Greenough divided his 73 cases of carcinoma of the breast into 3 groups, of low, medium and high malignancy. Of the 19 carcinomas belonging to group I, 82 per cent were cured, including 4 with involvement of the axillary lymph glands. In the third group, 16 cases had axillary deposits and 5 had none; all patients belonging to this group died of cancer. The author felt justified in considering the determination of the degree of malignancy to be reasonably accurate and to be of prognostic value in cancer of the breast.

Siemens grouped 67 cases of carcinoma of the breast as carcinoma solidum, scirrhus, adenomatous and medullary carcinoma. There were many transitions, especially between the first, second and third groups. The metastases in the lymph glands, which were present in 74 per cent of all cases, were mostly of medullary character, and only occasionally did they represent scirrhus or adenomatous structure. Siemens found the best operative results in adenomatous carcinoma and the poorest in the medullary type, but in his opinion the histologic analysis of a tumor of the breast does not permit a reliable clinical prognosis. Also Reinecke concluded from a comparison of the histologic types after healing among 99 amputations of the breast that the morphologic variation of the malignant epithelial proliferation is of relatively little prognostic significance. Still more skeptical was Reimann after studying 100 carcinomas of the breast. He based the histologic classification on the size and staining reaction of the cancer cells, the proportion of mitoses, the architecture of the tumor, the invasion of lymphatics and blood vessels and the reaction of the stroma. The prognosis thus arrived at was compared with the actual malignancy as determined by a follow-up of the patient. It proved to be wrong in 50 per cent of the cases. Reimann therefore contended that a prognostic classification based on mathematical grading of histologic characteristics is not much better than the impression of the degree of malignancy that the experienced pathologist receives from his usual histologic examination.

Van S. Smith and Bartlett studied 234 cases of malignant tumors of the breast. They believed that the determination of the degree of malignancy is most difficult at best, for it is rare to find a mammary tumor the cells of which are homogeneous throughout. Usually pictures of varying malignancy can easily be found in the same tumor, and frequently a metastasis has an entirely different appearance from that of the primary growth. Late recurrences may not have the slightest resemblance in degree of malignancy to the primary tumor.

Williams, Lange, Kaufmann and Ewing agreed as to a lower degree of malignancy of colloid cancer of the breast, while others like Depres.

Bertrand and de Nagy stated that the presence or the absence of mucus is not a factor in the postoperative prognosis. According to Cheatele and Cutler, the clinical course of tumors exhibiting gelatinous degeneration is determined chiefly by the properties of the epithelial elements which they contain and does not depend on either the presence or the extent of the colloid material. Four of their cases of colloid cancer of the breast were among the most malignant that can be encountered. In examining whole sections of the removed breast, they were able to discover gelatinous degeneration in tumors in which, from gross appearance, its presence was unsuspected.

Lee and Stubenbord were of the opinion that cases of carcinoma of the breast may be graded from purely clinical data more accurately than the histologic study can reveal. In 100 primary operable cancers of the breast, the authors estimated the degree of malignancy according to 4 clinical features, namely, age, presence of lactation, rate of growth and extent of the disease. Of their patients grouped in grade A, 69 per cent were alive and well after five years; group B showed 34 per cent and grade C 4 per cent surviving the five year period. This study is of especial interest because the same 100 cases were graded by Ewing solely on histologic criteria; 52 per cent of the patients who presented his grade I tumors were without evidence of the disease five years after operation, while among those with tumors of grade II and grade III there were 33 and 29 per cent, respectively, surviving the five year period. The divergence between the different histologic groups should be much wider to have the histologic grading of any significance. The tumors of 10 patients remaining well and alive were classified in the clinically benign group, but on a histologic basis these were placed in grade II. Of the highly malignant clinical group of cancers 9 in patients who succumbed to the disease within one year were classified as grade II on histologic criteria. It seems fair to conclude that the clinical grading made a more accurate selection of the patients than did the microscopic method.

To exclude the possible influence of differences in treatment on the clinical course, Daland made a study of untreated mammary cancer in 11 cases which came to autopsy. With the assistance of Greenough, the tumors were graded according to their histologic malignancy. None could be placed in group I; 6 were grouped in the second and 4 in the third group. Daland was unable to demonstrate a slower clinical course of the disease in the cases of lower potential malignancy. The location of the tumor appeared to him of greater significance in determining the length of life than the degree of histologic malignancy.

Snoke observed that cutaneous cancer of the face, in different locations, pursues a course independent of the cell type, and that anatomic factors, especially underlying bone, cartilage or fascia, may increase

the malignancy of a tumor regardless of cell differentiation. In his opinion, the histologic picture is only one aspect of the disease and at present an overemphasized one.

From his study of 319 cases of buccal carcinoma, Phillips concluded that a well marked correlation exists between the histologic character and the clinical course of buccal carcinoma, and that the usefulness of Broders' system of histologic classification has been amply demonstrated. Also Jorstad, after comparing histologic malignancy and clinical course in 200 cancers of the lip and oral cavity, felt that grading of carcinoma is of enough significance for its practice to be continued. The differences of longevity between Jorstad's histologic groups, especially his first, second and third, seem to me not so impressive as to justify his confidence in histologic grading; there were five year cures in 80 per cent of the first group, 76 per cent of the second and 60 per cent of the third. The clinicians of the Memorial Hospital, New York, judged that the histology does not aid in the selection of the treatment for cancer of the lip to the same extent that it does for cancer of the oral cavity proper, and that recognition of the clinical type of disease is of relatively greater importance. Neither did Fischel believe that as yet the histologic form of either the primary tumor of the lip or the metastases should influence the plan of surgical treatment. He would apply the most thorough local destruction of the tumor, followed by an equally thorough dissection of the neck, no matter what the histologic grade may be.

The periods of survival after the first symptoms which Clayton observed in 39 cases of squamous cell carcinoma of the esophagus do not place any significance on histologic classification of tumors in this location. The duration of disease after onset of the first clinical signs was, in his first group, sixteen months; in the second group, eight months; in the third group, five months, and in the fourth group, three and one-half months.

Grading according to morphology seems to apply fairly well to epithelial tumors of the larynx. A series of Thomson's cases of laryngeal carcinoma gave, in group I, 6 per cent, and in group IV, 72 per cent recurrences.

Konjetzny studied the end-results of gastric resection, to determine the relation of the histologic picture of carcinoma of the stomach to the clinical prognosis. He did not obtain decisive results, as there does not exist, in his opinion, a nomenclature for all the various histologic types of gastric carcinoma; furthermore, mixed forms of cancer occur frequently, the grading of which will differ widely according to whether only a single small portion of the tumor is examined or the whole specimen. Konjetzny arrived at the conclusion that there is little prospect of being able to determine with certainty the grade of malignancy of a given gastric carcinoma from the histologic picture

alone, and that the gross behavior of a gastric tumor is of greater significance. Also Brodnitz, Finsterer, von Haberer and Weil were unable to find any definite relationship between cell type and clinical prognosis in gastric carcinoma, while Scott expressed the belief that a slightly longer survival of the group showing the greatest cellular differentiation can be demonstrated.

Rankin and Broders graded 598 cases of carcinoma of the rectum in accordance with the principle of cell differentiation. The percentage of cases in which metastases were present rose in direct proportion to the grade of malignancy. The percentage of good results after operation decreased in inverse proportion to the grade of potential malignancy, and in cases of carcinoma graded III and IV without demonstrable metastases, the end-results were not much better than in cases of carcinoma of grade I that were accompanied by metastases. In colloid carcinoma of the rectum, Rankin and Chumley found a tendency of the amount of mucus present to be inversely proportional to the grade of malignancy, except in the cases of highest malignancy in which 58.3 per cent showed the highest observed grade of mucus formation. The authors admitted that this fact is not in accord with the view that mucus is a sign of cell differentiation. Furthermore, they found the clinical prognosis in colloid carcinoma unfavorable regardless of the grade of malignancy or the amount of mucus present if the lymph nodes were involved. Miles stated even that colloid carcinoma of the rectum is extremely malignant and is apt to recur after the most radical operation.

Bell regarded the two-stage abdominoperineal operation as the method of choice in rectal carcinoma when the grade of malignancy is high and when the physical condition of the patient permits this serious surgical intervention. Carcinomas of grades I and II, when near the anal margin, can be safely dealt with by colostomy and posterior resection, with an expectancy of life comparable to that offered by the abdominoperineal method without its high operative mortality.

Aschner did not believe that in tumors of the bladder prognosis can be made correctly from biopsy material and classification be based safely on cell grading alone. He regarded the presence or the absence of infiltration of the wall to be a more reliable guide to the gravity of the situation. The site of the malignant tumor also influences prognosis materially, since it determines its resectability. Hunt's observations were based on 370 treated patients with carcinoma of the bladder: Irrespective of the size and situation of the tumor or the magnitude of the operation, 65 per cent of those who had carcinoma of grades I and II lived three and more years without recurrence, as compared with 34 per cent of those who had carcinomas of grades III and IV. When the results were determined according to the sites of the tumors, he found

that those following operations on the base of the bladder were 50 per cent worse than those following operations for lesions in the lateral wall and dome of the bladder, irrespective of the grade of histologic malignancy.

According to Kolodny, the attempts of pathologists to form the prognosis of a tumor from its morphologic features alone has nowhere failed more than in tumors of the bone. The histologic structure is merely a link in the whole chain of circumstances that render a tumor malignant. In this chain much depends on purely clinical evidence. Age, rapidity of growth, situation, size of the tumor, encapsulation and vascularity are of the greatest importance in making a valuable prognosis in a given case.

Phemister presented 5 cases of undifferentiated round cell sarcoma of bone that proved to be exceptions to the generally accepted teaching that the less differentiated the cells the more malignant the tumor. Cures were obtained in all these cases, lasting from four and two-thirds to ten and three-fourths years.

The histologic grading of tumors of the brain is, according to Bailey and Cushing, of the greatest practical value. The cerebral gliomas, which represent about 42 per cent of all intracranial new growths, have heretofore been generally regarded as hopelessly malignant tumors. Impressed by the fact that many patients with tumors of this sort have survived for unexpectedly long periods of time after incomplete extirpation of the lesion, Bailey and Cushing undertook a reclassification of their cases, and they adopted for the gliomas a terminology consistent with that used for the histogenesis and normal structure of the brain. They subdivided their 254 gliomas into 13 categories, and found that the tumors in which the cells reproduced the structure of the less differentiated cells in the developing nervous system were more actively growing than were the ones composed of more highly differentiated cells. Almost half of their gliomas were of the latter sort and may be regarded—apart from the mischance of their occupying an inaccessible position in the brain—as comparatively benign lesions.

The successive groups of Cushing's newly classified gliomas when arranged in series according to the average periods of survival of the patients show a variation from those that leave the patient few months of life to others that, when properly treated, permit the patient to survive ten years or more. And this lengthened period goes hand in hand with the increasing degree of differentiation of the neoplastic cells. Bailey and Cushing concluded that no less important than the localization of tumors of the brain and technical methods is a clear understanding of the life history of the lesions treated, because on this more than on anything else depends the nature of the surgical intervention appropriate to the particular kind of tumor that happens to be disclosed.



In spite of the large volume of work that followed the pioneering of Broders, it must be said that the problem of histologic grading of cancer is far from being solved, and that its practical value is still very limited. The present knowledge of the relationship between histology and prognosis has been well summarized by Barnard: "A competent histologist can say of a tumor that it is a type which grows rapidly or slowly, and he may be able to add that it usually produces metastases early or late in its growth. But it is not yet within his power to draw a graph of malignancy indicating the expectation of life to be associated with each shade of variation to be found in malignant tumors."

#### IV. HISTOLOGIC CHARACTER AND RADIOSENSITIVITY OF TUMORS

Modern radiotherapy of cancer has its scientific foundation in the theory that neoplastic cells have a greater radiosensitivity than resting tissue cells. Cancer cells are thought to vary in their radiosensitivity in much the same way as normal cells, but knowledge of the relation between histologic character and response to irradiation is still rudimentary.

Perthes (1903) was the first to associate the radiosensitivity of a tissue with its reproductive activity, basing his belief on clinical observations and experimental studies. In 1906, Regaud and Blanc deduced from their experiments with testicles that in the life of a cell the stage of karyokinesis is the time of least resistance to irradiation. Later in the same year, Bergonié and Tribondeau from their observations of human tumors and experiments with rat testicles derived the general law that the greater the reproductive activity of cells, the more prolonged their process of karyokinesis and the less definitely fixed their morphology and their functions, the more intense is the action of the x-rays on them.

Recent histologic observations on irradiated tumors seem to substantiate the much defended view that irradiation acts by direct destruction of cells. Waetjen studied 4 squamous cell carcinomas of the uterine cervix in patients who died from one to six days after the application of radium. The earliest process noted after irradiation was a cessation of mitosis in the tumor cells; then the nuclei of the cells revealed evidences of degeneration, and finally the entire cells became necrotic. The stroma reaction—infiltration with leukocytes—appeared to be secondary to the changes in the tumor parenchyma. Gambarow also noticed in irradiated tumors primary degenerative changes in the cancer cells and secondary proliferation of the connective tissue. The nuclei in resting cells showed much less intensive changes than occurred during cell division. On 200 specimens of tissue obtained for biopsy in 30 cases of cervical carcinoma before, during and after irradiation, Fedorejeff was able to corroborate these observations. In the nuclei of the cancer cells

swelling, vacuolization, karyorrhexis and karyolysis occurred on the first day. Later the mitoses disappeared, but reappeared after two or three days in degenerated form, only to disappear completely after the fifth day. These nuclear changes were followed by swelling, vacuolization, hyalinization and lysis in the cytoplasm, leading to complete necrosis of the tumor cells. Inflammatory changes in the stroma and scar formation, hyaline degeneration and thrombosis of blood vessels were constant findings in the later stages.

Physiologic studies on tissue cultures by A. Fischer suggest that by irradiation growth-restraining substances are liberated from the cells. The effect of irradiation on resting cells was latent, manifesting itself only after the cells were forced to cell division.

When one attempts to apply the laws of radiosensitivity clinically, a definition of radiosensitivity is necessary in the first place. Two distinct phenomena are involved that do not parallel each other, namely, the primary regression of a tumor following irradiation and the definite cure. A thorough study of lymphosarcomas of the neck by Spies indicates that the more prompt and the greater the primary reduction of the irradiated tumor, the less time will the patient survive. In all of his 261 cases followed to the end, there was a probable mortality of 100 per cent. Lymphosarcomas, however, are generally regarded as models of radiosensitivity. By a definite cure is usually meant one that has lasted for at least five years. The published statistics of radiotherapy in which five year cures are related to morphologic characters of tumors are few. Furthermore, attempts to classify cancers from biopsies of small pieces of tissue into more or less radiosensitive groups are often fallacious, because examination of a small specimen obtained from the surface may not reveal the histologic characteristics of a given tumor varying in its different parts.

Ewing observed variations in grade of potential malignancy and in radiosensitivity in tumors from all parts of the body, less marked in tumors of the skin than in those of mucous membranes. According to him, response to irradiation usually runs parallel with the degree of anaplasia and potential malignancy, but by no means always. For instance, in the skin, basal cell carcinoma is relatively benign, but radiosensitive, while melanoma is highly malignant, but very radioresistant. Although in tumors of lip and tongue considerable variations in clinical malignancy occur, with corresponding changes in structure, most of these carcinomas are resistant to irradiation. The desmoplastic property of many epidermoid carcinomas is one of the most important factors in rendering them resistant. As a rule the more adult the cell type, the more active is the stroma reaction. On the other hand, the highly anaplastic and rapidly growing cellular tumors excite little stroma reaction and may be completely destroyed by external irradiation. Vascularity is

regarded by Ewing as one of the most important factors in the destruction of tumors by irradiation. Bulky papillary carcinomas melt away rapidly, mainly because the delicate blood vessels are destroyed. The location of the tumor has an important influence on the result of irradiation. In bone it is extremely difficult to destroy epidermoid carcinoma, because the inflammatory reaction is poor; fat tissue also is a very unfavorable bed for the treatment of most carcinomas. Ewing presented the factors entering into radiosensitivity in a table, which offers a provisional estimate of their importance. He listed as the most significant: embryonal quality and anaplasia of the cells, desmoplastic property, texture of the bed of the tumor and general condition of the patient. Also Haagensen emphasized that the constitution of the patient, local conditions and factors inherent in the tumor must be considered in an estimation of radiosensitivity, and that the phenomenon of radiosensitivity is a great deal too complex to be envisaged by a simple statement of one or two factors.

Epidermoid carcinomas are, in Regaud's opinion, the only epithelial tumors that are cured by irradiation almost as a routine. Since the time of Darier (1904) it had been believed that carcinomas of the skin could be divided into radiosensitive basal cell carcinomas and resistant squamous cell cancers. Regaud found that the sensitivity of both varieties of cancer is the same; similar end-results of treatment of the basal cell and squamous cell types by the same technic showed that the older view was incorrect. This fact does not contradict, however, the experience that it is more difficult to obtain a clinical cure in squamous cell carcinoma than it is in basal cell cancer. In the former, the early invasion of the deeper tissues makes it much more difficult to reach all involved parts, whereas in the latter the entire disease is usually superficial and of slight extent.

The most frequently quoted paper supporting Bergonié's law that the radiosensitivity increases with the degree of immaturity of tumor cells is the study by Healy and Cutler. They distinguished among 200 cases of carcinoma of the cervix 3 groups: (1) the adult type with hornification and occasional pearl formation; (2) the plexiform type with large, partially differentiated tumor cells and (3) the anaplastic type with small round or spindle cells. In advanced cancer of the cervix the end-results after radiotherapy were extremely poor when the neoplasm was of the adult type and unexpectedly favorable when it was of the anaplastic type. In the early stages of the disease, the cures were comparatively high, regardless of cell type. Healy and Cutler concluded from their studies that the clinical stage at which the treatment is instituted and the type of the cancer cells are the most important factors in clinical prognosis when radiation is employed.

Bowing and Fricke stated that their experience with 625 cases of cervical cancer is in accord with the view of Healy and Cutler, but, in my

opinion, their statistical data are not convincing in regard to the prognostic value of histologic grading. The rate of survival of patients with tumors of their grade II was 52.9 against 54.99 per cent of those with tumors of grades III and IV. Thibaudeau and Burke concluded from their investigation of 28 cases of carcinoma of the cervix in which the patients were alive and well five years after roentgen treatment, that histologic grading does not give any information about radiosensitivity.

Schmitz and Hueper also were unable to demonstrate the validity of the law of Bergonié in tumors of the uterine cervix and of the breast. While the primary effect of irradiation on highly immature tumors may be grossly more marked than that seen in well differentiated carcinomas, the permanent curative results obtained in undifferentiated cancers were poorer than those seen in the mature types. Schmitz and Hueper expressed the belief that the degree of histologic malignancy is of the same significance in regard to prognosis in radiotherapy as it is in surgery.

Doederlein classified 275 irradiated cervical cancers into undifferentiated, moderately differentiated and well differentiated histologic groups. The degree of differentiation among the squamous cell carcinomas played practically no rôle in their response to irradiation. According to Lacassagne, the radiosensitivity of an epidermoid cancer of the uterine cervix cannot be forecast from a consideration of the size and form of the tumor cells, the number of mitotic figures, the stroma reaction or the condition of the blood vessels. Histologic observations do not explain, in his opinion, why some very extensive cancers of the cervix can be cured in spite of insufficient roentgen treatment, while others of very limited extent recur in spite of proper treatment.

In buccal carcinoma, Phillips did not find any direct relationship between histologic character and response to irradiation; the highly differentiated carcinoma reacts to radiotherapy as well as the very anaplastic types. Quick and Widmann, on the other hand, stated that in this location radiosensitivity depends on cellular differentiation. Stewart found cancers of the oropharynx more radiosensitive than those located in the anterior portion of the buccal cavity, in spite of similar histologic structure. Rather adult epidermoid carcinoma, situated in the pharynx, may be as radiosensitive as the rapidly growing anaplastic lympho-epithelioma of this region (Haagensen). Lacassagne's observations also signify that in oral cancer the clinical cure depends not less on the anatomic site of the tumor than on the histologic character. His end-results in cancer of the floor of the mouth were poorer than those obtained in carcinoma of the tongue, in spite of the fact that the former was composed of more immature cells.

There seems to be general agreement that adenocarcinomas of different organs are not amenable to irradiation. This failure of roentgen

therapy is apparently due not only to difficulty in reaching the deeper parts of the tumor and to early invasion of regional lymph glands, but to inherent radioresistance. Regaud explained this resistance by the biologic fact that, in general, a cell is scarcely, if at all, radiosensitive when it is in secretory activity. The difference in radiosensitivity of epidermoid and glandular carcinoma was clearly demonstrated by Windholz. He had the opportunity to examine histologically a uterus in which a squamous cell carcinoma and an adenocarcinoma were situated close together. While the epidermoid carcinoma showed marked degeneration, the glandular tumor was only slightly affected by irradiation. Regaud stated that he had never succeeded in curing a case of adenocarcinoma of the uterus by short wave irradiation. Also the sensitivity of rectal adenocarcinoma is generally feeble; the few authentic cures of rectal carcinoma have apparently been obtained by methods of diffusely caustic radiotherapy. In interesting contrast to Regaud's statement are the observations of Bowing and Fricke that adenocarcinomas of the uterine cervix of all four histologic grades appeared to have a better prognosis after irradiation than the squamous cell types. In Doederlein's experience, the glandular carcinomas of the corpus were more radiosensitive than those of the cervix. Eleven of his 17 patients were alive and well after five years, and the histologically riper forms were associated with a higher rate of survival than the unripe forms.

Owing to the confusion in the classification of the various forms of mammary cancer, the knowledge of their radiosensitivity is extremely meager. According to Lacassagne, certain cases are amenable to radiotherapy while others withstand the most vigorous irradiation, and yet up to the present histologic differentiation between these two varieties is unsuccessful. Metastases of mammary carcinoma in lymph glands and recurrences in the skin disappear often promptly after irradiation, while the primary tumor of the breast does not respond to this form of treatment. It seems probable that the reaction of tumor tissue to irradiation depends as much on such factors as blood supply and character of the stroma as on the inherent qualities in the cancer cell itself (Lee). Pfahler regarded histologic grading of tumors of the breast as of no aid in the determination of the prognosis when irradiation is employed.

As a result of unsatisfactory results of surgery in cancer of the thyroid gland, irradiation is more and more applied in the treatment of these tumors. Haagensen and Clute and Warren agreed that the less rapidly growing and less anaplastic types, especially adenocarcinomas, are moderately radiosensitive, while the highly anaplastic types are not affected by irradiation. In this particular group of tumors, the law of Bergonié and Tribondeau has therefore no validity.

Reviewing the literature on histologic character and radiosensitivity of tumors, I can only repeat the statement of Plaut in 1927, that there

is a bewildering mass of contradictions and misunderstandings in a matter of greatest theoretical and practical importance. Further knowledge must come through painstaking analysis of particular groups of tumors. As Wood pointed out at the Cancer Symposium of the Radiological Society in 1930, the great clinics of this country, which have a large choice of material at their disposal, owe to the medical profession, and through it to humanity, the publication of mass statistics that will include not only complete clinical histories and five year results, but also photomicrographs of all individual types of cancer. Such a complete and detailed atlas, demonstrating the relation between morphologic characteristics and radiosensitivity on several thousand tumors of one particular kind, would be appreciated by pathologists throughout the world as a real contribution to the ultimate conquest of cancer.

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# Notes and News

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## DOCTORATES IN BACTERIOLOGY AND PATHOLOGY GRANTED BY AMERICAN UNIVERSITIES, 1931-1932

Clarence J. West and Callie Hull, Research Information Service,  
National Research Council, Washington, D. C.

### BACTERIOLOGY

Brown: Merrill Wallace Chase, "Further Studies on the Liberation of Toxins from *Salmonella Schotmuelleri* Principally by Repeated Freezings and Thawings." Robert Merrett Pike, "Toxic Substances Produced by *Staphylococci* Grown on Solid Media with Special Reference to the Depression of Phagocytosis by *Staphylococcus Filtrates*."

California: Claude E. ZoBell, "Cultural Requirements and Metabolism of the *Brucella* Group."

Chicago: William Burrows, "The Nutritional Requirements of *Clostridium Botulinum*." Leo Philip Doyle, "I. Serum Reactions Produced by Feeding Antigens. II. The Length of Survival of Paratyphoid Bacilli in Foodstuffs." Carl Oswald Lathrop, "Biological Studies of Pathogenic Strains of *Bacterium Coli* from Human Sources." Einar Leifson, "Bacterial Spores." Bertha Kaplan Spector, "A Comparative Study of Cultural and Immunological Means of Diagnosing Infections with *Endamoeba Histolytica*."

Columbia: Margaret Holden, "The Nature and Properties of the Virus of Herpes."

Cornell: Herman Jacob Brueckner, "Primitive or Filtrable Forms of Bacteria and Their Occurrence in Milk." Clifford Darton Kelly, "Chemical Changes in Cheddar Cheese in Their Relationship to the Lactic Acid Streptococci." Clair Eugene Safford, "The Occurrence and Numbers of Primitive Forms of Bacteria in Nature and in Pure Culture." Abdel Magid Wahby, "Growth of Pathogenic Bacteria in Milk."

Iowa State College: Dean Albert Anderson, "The Production of Gum by Certain Species of *Rhizobium*." Harry E. Goresline, "Studies on Agar-Digesting Bacteria." Ralph Victor Hussong, "The Relationships of a Lipolytic Organism to Rancidity of Butter." John Albert Nelson, "The Correlation Between the Organisms Found Microscopically and the Bacterial Deterioration of Butter." Harold Cecil Olson, "The Microflora of Churns and Its Importance in the Deterioration of Butter." Roger Patrick, "The Fermentation of Levulose by Some Bacteria of the Genus *Aerobacillus*." Arthur Wesley Young, "The Winogradsky Spontaneous Culture Method for Determining Certain Soil Deficiencies."

Johns Hopkins: Eric Christian Gilles, "The Isolation of Tetanus Bacilli from Street Dust." Eloise Elaine Greene, "A Study of Spirochetes in the Cat, with Special Reference to Those of the Alimentary Tract."

Massachusetts Institute of Technology: Marshall Walker Jennison, "A Critical Study of the Effect of Temperature upon Certain Aspects of Bacterial Growth Curves."

Michigan: Alden Franklin Roe, "Anaerobic Methods and the Dissociation of *Bacillus Welchii*."

Minnesota: Millard Fillmore Gunderson, "Studies on Western Duck Sickness." Milan Vaclav Novak, "Dissociation of an *Actinomyces* into Bacterial Forms."

Ohio State: Eva Galbreath Campbell, "A Thermophil Nitrite Former."

Pennsylvania: Bess E. Segal, "Serological and Cultural Studies of Meningococci with Special Reference to Type V."

Pittsburgh: Felice A. Rotondaro, "The Development of Bacteriology: Bibliography."

Rochester: Clarence Frederic Schmidt, Jr., "Studies in Bacterial Calorimetry."

Rutgers: Herbert W. Reuszer, "The Decomposition of Hemicelluloses with Special Reference to Their Uronic Constituents."

Vanderbilt: Lurline Valeria Richardson, "Diphtheria Antibodies Transmitted to the Offspring of Immune Guinea Pigs."

Washington: Ruby May Bohart, "A Study of Sensitization in Experimental Tuberculosis."

Wisconsin: Lois Almon, "The Stability of Cultures of *Rhizobium*." Ruby Bere, "I. Copepods Parasitic on Fish of the Trout Lake Region. II. The Effect of Freezing on the Number of Bacteria in Ice and Water from Lake Mendota. III. The Bacterial Content of Some Wisconsin Lakes." Adolph Alexander Hendrickson, "Studies on Certain Physiological Characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes* and *Bacillus radiobacter* and Their Relations to Plants." Elmer Reeve Hitchner, "A Cultural Study of the Propionic Acid Bacteria." Ernest Carr McCulloch, "The Germicidal Efficiency of Sodium Hydroxide." Harry Edwin Sagen, "Comparison of Certain Physiological Characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes* and *Bacillus radiobacter*." William Bowen Sarles, "The Production of Volatile Acids by the Fermentation of Cellulose at High Temperatures." Chun Chieh Young, "Oxidation and Reduction of Alexin."

Yale: Agnes Roseburgh Beebe, "The Influence of Age upon the Reaction to Toxins, Especially in the Neonatal Period." Catherine Seeley Flynn, "On Variation and Filtrability of *Bacillus mesentericus* and *Bacillus vulgatus*." Norman Edwin Gibbons, "A Bacteriological Study of Fresh and Frozen Marine Fishes." Lawrence William Slanetz, "A Systemic Study of the Fusiform Bacteria."

#### PATHOLOGY

Chicago: Cornelius Albertus Hospers, "Studies in Cholesterol Metabolism: I. The Experimental Production of Gallstones in Hypercholesterolemic Rabbits. II. The Blood Cholesterol in Anesthesia." Robert Stewart Jason, "Studies on the Histopathology of the Palatine Tonsil and on the Immunological Reactions of the Adjacent Tissues." Guillermo Alfredo Pacheco, "Cellular Immunity." William Taylor Miller, "The Blood Picture in Healthy Cattle and Cattle Affected with Johne's Disease."

George Washington: Leon Stuart Gordon, "The Pathology of Intracranial Hemorrhage in the New-Born."

Johns Hopkins: Meridian Ruth Greene, "The Effects of Vitamins A and D on Antibody Production and Resistance to Infection." Pearl Kendrick, "The Antigenic Properties of Bacteriophage Lysates of *Salmonella suipestifer* as Indicated by the Antibody Response in Rabbits."

Michigan State College: Leo Hansom Himmelberger, "The Significance of Lymphatic Tissue and Adenoma-Like Areas in the Thyroid Gland."

New York: Julius August Klosterman, "Studies on Experimental Spirochaetosis Icterohaemorrhagica (Weil's Disease)."

Northwestern: Dora Fishback, "Alteration of Glycogen, Lactic Acid and Phosphorus Compounds in Experimentally Produced Acute Molecular Degeneration of Skeletal Muscle."

Stanford: Denis Llewellyn Fox, "Some Chemophysical Aspects of Carbon Dioxide Narcosis in Living Cells."

Washington University, St. Louis: James Ralph Wells, "The Origin of Immunity to Diphtheria in Isolated Communities of Central and Polar Eskimos."

Western Reserve: Lyle Alfred Weed, "The Biological Properties of Certain Organic-Mercury Salts."

Yale: Leona Baumgartner, "Qualitative Changes in Antibody During the Development of Serological Maturity."

**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Hugh K. Ward has been appointed assistant professor of bacteriology and immunology in Harvard Medical School.

Cyril N. H. Long, assistant professor of medical research in McGill University, has been appointed director of the Cox Institute for a Research in Diabetes at the hospital of the University of Pennsylvania.

John A. Kolmer has resigned as professor of pathology and bacteriology in the graduate school of medicine of the University of Pennsylvania and has accepted the professorship of medicine in Temple University, Philadelphia.

Sir Ronald Ross, the discoverer of the transmission of malaria by mosquitoes, died in London on Sept. 16, 1932, at the age of 75.

**Society News.**—The forty-third annual meeting of the Association of American Medical Colleges will be held in Philadelphia on Nov. 14 to 16, 1932.

The thirty-fourth annual meeting of the Society of American Bacteriologists will be held at Ann Arbor, Mich., Dec. 28 to 30, 1932.

The next meeting of the Pathological Society of Great Britain and Ireland will be held at University College Hospital Medical School, London, on Jan. 6 and 7, 1933.

# Abstracts from Current Literature

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## Pathologic Anatomy

CARCINOMA OF THE GASTROJEJUNAL STOMA FOLLOWING OPERATION FOR PEPTIC ULCER. HARRY A. SINGER, Arch. Int. Med. **49**:429, 1932.

The reappearance of symptoms following gastro-entero-anastomosis for peptic ulcer generally indicates a benign complication or sequel. Recurrence of the old or formation of a new ulcer and nonmalignant obstruction are most frequently suspected. Occasionally, however, the clinical manifestations may be produced by a carcinoma. In one such case, that of Schwarz, a patient who had remained well for six years after gastro-enterostomy began to have symptoms of obstruction of the stoma, which were due to a malignant growth. In another case, that of Eichelter, a neoplasm by encroaching on the lumen of the stoma led to manifestations referable to obstruction of the proximal loop. In a third case, the one herein reported, the carcinoma, being polypoid, produced no clinical obstruction, but gave rise to a set of indistinctive symptoms. The presence of a palpable mass and the roentgen evidence of a filling defect within the gastric silhouette furnished the chief reasons for the preoperative diagnosis of carcinoma of the anastomosis.

FROM THE AUTHOR'S SUMMARY.

SQUAMOUS EPITHELIAL RESTS IN THE HYPOPHYSIS CEREBRIS. H. T. CARMICHAEL, Arch. Neurol. & Psychiat. **26**:966, 1931.

The normal hypophyses removed at necropsy in a group of fifty-five cases were studied by means of serial sections. Masses of cells resembling squamous epithelium in histologic appearance were found in eighteen cases (32.7 per cent). In the nine subjects who were less than 20 years of age, groups of cells resembling squamous epithelium were not found. The incidence of these masses of cells, which apparently were squamous epithelium, among the forty-six adult subjects was 39.1 per cent. It seems reasonable to attribute the origin of these groups of cells, which apparently were squamous epithelium, to rests of the former hypophyseal duct, and to accept their relation to the so-called tumors of the hypophyseal duct; that is, if it is accepted that the rests cannot be identified in subjects who have not reached the age of 20 years, because at that time the cells of the rests are not sufficiently differentiated from the other epithelial cells in the neighborhood.

AUTHOR'S SUMMARY.

THE HISTOPATHOLOGY OF TRIORTHOCRESYL PHOSPHATE POISONING (GINGER PARALYSIS). MAURICE I. SMITH and R. D. LILLIE, Arch. Neurol. & Psychiat. **26**:976, 1931.

The nerve changes caused by drinking adulterated Jamaica ginger have been studied by Smith and Lillie on animals (monkeys, cats, dogs, chickens) and the results compared with those found in men. The animals were given orally, subcutaneously or intravenously various doses of triorthocresyl phosphate and were kept alive from several hours to about two months. The peripheral nerves, spinal cord and viscera were studied with numerous methods. The main changes were found in the peripheral nerves with especial involvement of the myelin; much less affected were the motor cells of the spinal cord. The lesion of the cord is considered secondary, the result either of axonal reaction or of extension of the degeneration from the peripheral nerves. The conclusions are that triorthocresyl phosphate is essentially a poison of the nervous system affecting mainly the lower motor neuron.

GEORGE B. HASSIN.

CHRONIC PROGRESSIVE SUBCORTICAL ENCEPHALITIS. FREDERIC J. FARNELL and JOSEPH M. GLOBUS, *Arch. Neurol. & Psychiat.* **27**:593, 1932.

A woman, aged 40, after two falls complained of pain in the back and nervousness. Eleven months later, the patient was unable to walk, lost sensations in the legs and complained of pollakiuria. The patient showed involvement of the pyramidal tracts, marked flexion of the upper and lower extremities and, in the later course of the disease, a definite picture of decerebrate rigidity. The course was rapid and progressive. Microscopic examination revealed a normal cortex, degeneration of the subcortex with foci of demyelination, microglia and fat granule bodies filled with fat, arteriosclerosis and "almost complete general disintegration" of the blood vessels. Fat changes, "definitely pathologic," of the ganglion cells of the basal ganglions, substantia nigra and pyramidal tracts were present. In the presence of arteriosclerotic changes, the disease in question differs from Schilder's disease.

GEORGE B. HASSIN.

HISTOLOGIC CHANGES IN THE BRAIN IN FATAL INJURY TO THE HEAD. CARL W. RAND and CYRIL B. COURVILLE, *Arch. Neurol. & Psychiat.* **27**:605, 1932.

The authors studied the reaction of microglia and oligodendroglia to injuries of the brain—edema, contusion, laceration and hemorrhages (gross and petechial). Twenty-four cases were studied, all in human beings, with special reference to the length of time that had elapsed between injury and death and to the type of lesion. In edema, the microglia is generally not affected. In contusion, the changes in the microglia depend on the severity of the injury and its age. They may occur as early as within one hour or less. The early changes are vacuolization, mitotic and amitotic division of cells and swelling of the major cytoplasmic processes. Transitional microglial changes up to the formation of fat granule bodies were found. The authors could not form conclusions as to the difference between clean puncture wounds in experimental animals and diffuse contusions following injury to the human brain. The general course of the microglial changes is: early swelling of the major processes, or a definite swelling of the cytoplasm and all the processes, the spider cell stage, the ameboid stage and the stage of compound granule corpuscles. In hemorrhages of the brain, the microglial reaction is delayed until the red cells are destroyed. The conclusion arrived at is that the microglia have a primary phagocytic function and probably a secondary limited protective reaction.

The reaction of the oligodendroglia may be local, near the injury, but is usually general, in contrast to the microglial reaction, which is local. The early reaction shows as acute swelling, more or less uniform, in all portions of the brain. It may be prompt. It was present in a woman killed outright, and in another case within thirty minutes after injury. If the injury is not severe, the cell may be restored. Regressive changes—pyknosis and vacuolation—occur often, especially near the place of injury. Transformation into fat granule bodies was not observed.

GEORGE B. HASSIN.

FAMILIAL PROGRESSIVE MUSCULAR ATROPHY. GEORGE A. MOLEEN, W. C. JOHNSON and H. H. DIXON, *Arch. Neurol. & Psychiat.* **27**:645, 1932.

One of three brothers afflicted with progressive muscular atrophy was followed for over twenty years. The main clinical feature was absence of the claw hand, though the thenar and hypothenar eminences were markedly wasted. Pathologic examination twenty-nine years after the onset revealed practically normal ganglion cells throughout the spinal cord, pons and other regions, but marked degenerative changes in the peripheral nerves and muscles. In the former, the myelinated fibers were decreased in number; many were replaced by connective tissue and an increased number of fibroblasts. The nonmyelinated fibers were intact. The muscle fibers showed atrophy, some more, some less. In advanced stages, only remnants of muscle fibers were left—in the form of granular material and

particles of a hyaline substance. In early stages, the nuclei, which were small and pyknotic, were increased in number, and in later stages they formed clusters. Only insignificant ganglion cell changes were found in the spinal cord; these were in the lumbar region, and were considered to be the result of retrograde degeneration.

GEORGE B. HASSIN.

CHRONIC PROGRESSIVE CHOREA. C. DAVISON, S. P. GOODHART and H. SHLIONSKY, *Arch. Neurol. & Psychiat.* **27**:906, 1932.

Three cases of chronic progressive chorea (two were of Huntington's chorea and one was due to arteriosclerosis) were studied clinically and pathologically. The gross changes were: arteriosclerosis of the brain, with especial involvement of the basal ganglions and cerebellum, and atrophy of the convolutions of the brain and of the corpus striatum. In one case, the globus pallidus was shrunken. There was acute and chronic cell disease in the third, fifth and sixth cortical layers, involving small and to a lesser extent large ganglion cells of the neostriatum. In one case, demyelination of the white fibers of the frontal and temporal lobes was present. The rostral portion of the caudatum was more involved than the caudal. The combination of cortical and subcortical (neostriatal) changes in the three cases speaks for a complex mechanism of the involuntary movements in progressive chorea.

GEORGE B. HASSIN.

LISSAUER'S DEMENTIA PARALYTICA. H. HOUSTON MERRITT and M. SPRINGLOVA, *Arch. Neurol. & Psychiat.* **27**:987, 1932.

In eight cases of atypical dementia paralytica (Lissauer's form) there occurred epileptiform and occasionally apoplectiform attacks, which were followed by hemiplegia and aphasia. Microscopic changes were present in the cortex, which showed outspoken atrophy not so much in the frontal as in the temporal and inferior parietal lobes. Changes were also present in the basal ganglions and the subcortical white substance. Of other microscopic changes, the authors point out status spongiosus (vacuolated defects of the gray and white brain tissue), which was especially marked at the apex of the convolution; laminar loss of the ganglion cells (in the second and third convolutions), and areas of demyelination (in the temporal lobe, supramarginal or occipital lobe convolutions). In some cases, the deeper laminae (fifth and sixth) showed swollen, balloon-like cells (in the third cortical layer) as seen in Pick's disease (atrophy of the brain), in addition to other nerve cell changes as described by Nissl. The myelin fibers exhibited marked destruction. Of the catabolic products, iron pigment was found in all the cases. The status spongiosus and the cortical atrophies are regarded as due, not to the paralytic process, but to a combination with a functional disturbance of circulation, as the distribution of the atrophy in these cases followed the branches of the middle cerebral artery.

GEORGE B. HASSIN.

SPONTANEOUS SUBARACHNOID HEMORRHAGE IN RELATION TO CEREBRAL ANEURYSM. I. STRAUSS, J. H. GLOBUS and S. W. GINSBURG, *Arch. Neurol. & Psychiat.* **27**:1080, 1932.

By "spontaneous subarachnoid hemorrhage" is meant massive extravasation of blood into the subarachnoid space from spontaneous rupture of a blood vessel. The common anatomic change is arteriosclerosis of the cerebral blood vessels, with or without aneurysm. Inflammatory lesions of blood vessels may also cause aneurysm followed by rupture and hemorrhage. Repeated lumbar puncture to relieve intracranial tension is indicated. Such hemorrhage may occur almost at any age, but is most frequent between the ages of 25 and 40 years.

GEORGE B. HASSIN.

BRAIN CHANGES IN MALIGNANT ENDOCARDITIS. I. B. DIAMOND, Arch. Neurol. & Psychiat. **27**:1175, 1932.

The lesions of the brain in thirteen cases of endocarditis were studied. The changes are circumscribed or diffuse, inflammatory or degenerative, usually multiple, and are classified by Diamond as nodules, abscesses, vascular lesions, degenerative lesions and attempts at repair. The nodular structure varies. It may be glial, glial-mesodermal, mesodermal or perivascular, glial-leukocytic, or exclusively leukocytic (miliary abscesses), or may show giant cells. Degenerative lesions with softening and necrosis may result from vascular disturbances. In a state of repair, the nodules are rich in capillaries, many newly formed and scattered among glia and microglia cells. The diffuse changes in the brain combined with those in the meninges make a picture of meningo-encephalitis. Both focal and diffuse lesions of the brain are to be looked on as manifestations of the defense reaction against infection and intoxication.

GEORGE B. HASSIN.

ENCEPHALITIS IN MEASLES. A. FERRARO and I. H. SCHEFFER, Arch. Neurol. & Psychiat. **27**:1209, 1932.

Ferraro and Scheffer studied two additional cases of encephalitis in measles. Perivascular, or rather extra-adventitial, proliferation of microglia and demyelination in the same areas are common features. The axons may also be involved. Microglial changes are less characteristic. The ganglion cells may show changes from simple swelling to complete degeneration. In general, the changes are similar to those in postvaccinal encephalitis. In the two cases, death occurred within thirty-six hours after the onset of the disease. There were diffuse, parenchymal lesions of the brain with involvement of all the lamina of the cortex, especially those of the frontal lobe. The basal ganglions and Purkinje cells were also involved. Oligodendroglia in both the cortex and the white substance showed diffuse swelling. Microglia were less involved, while the astrocytes showed both progressive and regressive changes. Marked proliferation of microglia and demyelination were absent. Instead, changes of the gray matter were prominent and resembled those occurring in experimental toxemias, for instance, lead poisoning. The differences from the changes usually described are due, they believe, to the time element, as the course was peracute, and the organism did not have time to develop the usual reactive phenomena.

GEORGE B. HASSIN.

STRUCTURAL CHANGES IN THE SKIN IN CHRONIC HEREDITARY EDEMA OF THE EXTREMITIES (MILROY'S DISEASE). J. MCGUIRE and P. ZEEK, J. A. M. A. **98**:870, 1932.

The skin of a boy of 15 years who had typical hereditary edema showed: normal epidermis, increased density in the dermal papillary layer, edema with increase in the collagen fibers in the reticular layer, scattered lymphocytic infiltration especially about the small veins and capillaries of the subpapillary network, increase in the subcutaneous tissue with edema, the fluid in places forming pools, and apparent hypertrophy of the arteriolar media.

HETEROTOPIC BONE FORMATION. H. WURM, Beitr. z. path. Anat. u. z. allg. Path. **85**:401, 1930.

From an investigation of heterotopic bone formation Wurm concludes that those calcific deposits the chemical composition of which is similar to bone ash undergo ossification. Cellular (foreign body giant cells with osteoclastic capabilities) as well as humoral forces resorb implanted calcium masses, and superficial lamellar ossification occurs through the activity of proliferated fibrocytes without any preceding cartilaginous stage. In addition, he recognized as a further prerequisite locally increased concentration of calcium ion, which he regards as the factor in the ossification of tendon and muscle following injury rather than displacement of osteoplastic tissue.

W. S. BOIKAN.



THE ANATOMIC DIAGNOSIS OF ALCOHOLOGENIC DISEASES OF THE BRAIN.  
K. NEUBÜRGER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:395, 1931.

The anatomic diagnosis of the effects of chronic alcoholism on the brain can be easily made in many instances by examination of the corpora mamillaria. Often there are characteristic macroscopic changes, namely, small brown discolorations and softenings of these structures. Microscopic study reveals almost always endarteritis of the small blood vessels, proliferation of the microglia and fatty degeneration of the glia cells, changes that do not appear to occur in other diseases.

C. ALEXANDER HELLWIG.

HISTOLOGIC FINDINGS IN SKELETAL MUSCLES FOLLOWING ELECTRIC SHOCK.  
S. T. GELLINEK, *Wien. klin. Wchnschr.* **45**:37, 1932.

Gellinek reports changes in muscles in two instances of sudden death as a result of electric shock. In the first case, a young man died after having come in contact with an alternating current of 380 volts. The individual muscle fibers close to the region where the electric current had entered the body contained distinct spirals, due to a peculiar arrangement of the sarcoplasm. The threads of the spirals appeared thick and dark without showing any recognizable detail; they were clearly noticeable in preparations stained with hematoxylin and eosin, but became more prominent when Heidenhain's technic was applied. They were regular, and the pitches were found at even intervals. The sarcoplasm between the threads of the spirals (constituting the pitches) showed the cross-striations clearly. The diameters of the muscle fibers in the region of the threads were enlarged as compared with the diameters of the pitches or with those of unchanged muscle fibers. The thickness of the threads measured from 13 to 19 microns. Similar changes were noted in the muscles in the second case. The patient had died suddenly after having been in contact with 5,000 volts of alternating current. In this instance, however, a distinct transverse striation was noted in the threads of the spiral. The myocardium was also examined, and similar spirals were found within the muscle fibers. These spirals were not so sharply outlined as the ones in the skeletal muscles, but the regularity of the threads was clearly noted. Gellinek was also able to produce such spiral-containing muscles experimentally in guinea-pigs after the application of an alternating current of 220 volts. Special emphasis is laid on the application of an alternating current. By the use of a direct current, spirals could not be produced. Gellinek notes that the histologic structures of the muscle fibers were apparently, if at all, only slightly altered. The formation of the spirals in the muscles seems a pure electro-energetic effect—according to Gellinek, the first ever noted in the biologic field.

O. SAPHIR.

THE CEREBRAL LESIONS OF DEMENTIA PARALYTICA IN COMPARISON WITH THE LESIONS OF A SPONTANEOUS DISEASE IN CHICKENS. FREDERIC WERTHAM, *Ztschr. f. d. ges. Neurol. u. Psychiat.* **136**:62, 1932.

In investigations of the effects of experimental infection of the chicken's brain with *Spirochaeta gallinarum*, in which a series of control animals was also examined histologically, a hitherto undescribed disease of chickens was discovered. The main histologic characteristics were: infiltration of the small blood vessels with plasma cells, marked proliferation of the Hortega glia, with formation of typical rod cells, and iron deposits in diffuse distribution in the intra-adventitial spaces of small vessels and, to a much less extent, in the microglia (Hortega cells).

In addition there were: alterations of nerve cells, in diffuse distribution as well as in special regions; small glia cluster, consisting for the most part of microglia, in the white matter and the gray matter of the cerebrum, the cerebellum and the spinal cord: slight infiltration of the meninges with plasma cells, and in some cases marked endymenitis of the ventricles.

The etiology of this spontaneous disease has not been determined. It is unlikely that a spirochete is responsible for the disease. Histoparasitologic examination with appropriate methods revealed no elements that had any similarity to known micro-organisms. Transmission experiments, which are being continued on birds free from this disease, such as canaries, have so far not led to positive results.

The demonstration of these lesions is significant for the following reasons: Iron deposits of the type described have been observed elsewhere only in dementia paralytica (with the exception of very small amounts in three cases of human trypanosomiasis). For this reason, this type of iron deposit was termed "dementia paralytica iron," a designation generally accepted by neuropathologists. The evidence that such iron occurs also in animals opens a way for further investigation of its significance.

The three outstanding histologic signs of this disease of chickens correspond to the three cardinal histologic characteristics of dementia paralytica, namely, plasma cell infiltration of small vessels, "dementia paralytica iron" and the formation of rod cells. The less significant histologic lesions of the spontaneous disease of chickens, such as changes of nerve cells, small glia clusters, ependymitis, etc., also occur in dementia paralytica. It may therefore be concluded that the histologic lesions of dementia paralytica are not specific, but constitute a reaction of the central nervous system that may occur under different circumstances.

It is likely that some of the lesions that have been described as characteristic of infectious and nutritional disorders in birds are in reality the effect of this spontaneous disease, just as the lesions of spontaneous encephalitis of rabbits have been taken for the effect of syphilis or epidemic encephalitis.

FREDERIC WERTHAM.

OCHRONOSIS IN CATTLE. H. FINK and W. HOERBURGER, *Ztschr. f. physiol. Chem.* **202**:8, 1931.

A case of ochronosis (osteohemochromatosis) in a cow is described. The bones were mahogany brown and showed in filtered ultraviolet rays the characteristic bright red porphyrin fluorescence. The enamel of the teeth, in contrast to the dentin, did not fluoresce. The myeloid and fat marrow did not show fluorescence. Analysis of the bones gave a relatively small amount of uroporphyrin octamethyl ester. For the identification of this substance the  $p_H$  fluorescence curve was used. The ground spongy bones of the pelvis, scapula, etc., gave a definite hemochromogen spectrum, but not a porphyrin spectrum, while the hemochromogen spectrum was absent in the long bones and only an alkaline porphyrin spectrum was present. The iron content of the bones was within normal limits. Urohematin could not be demonstrated.

WILHELM C. HUEPER.

DEATH FROM RUPTURE OF A TUBERCULOUS ANEURYSM OF AN INTERCOSTAL ARTERY. E. LINDHAGEN and E. KARLMARK, *Acta med. Scandinav.* **76**:88, 1931.

A case of fatal hemothorax from a tuberculous aneurysm of the fourth right intercostal artery in pleural tuberculosis is described. The bleeding took place gradually through a small opening that passed through tuberculous granulation tissue.

SCLEROSIS AND ANEURYSM OF THE PULMONARY ARTERY WITH PATENT FORAMEN OVALE. H. OKKELS and F. THERKELSEN, *Acta path. et microbiol. Scandinav.* **9**:214, 1932.

A man, 31 years old, had suffered from marked dysemia and cyanosis since childhood. After death there were found pulmonary arterial sclerosis with patent foramen ovale and an aneurysm of the pulmonary artery just above the valves. There was no defect in the septum, and the ductus botalli was closed.

## Pathologic Chemistry and Physics

DISTRIBUTION OF SUGAR BETWEEN BLOOD CELLS AND PLASMA. MARTIN JACOBY and HELENE FRIEDEL, *Biochem. Ztschr.* **244**:356, 1932.

Using the Hagedorn-Jensen method, the authors find that the corpuscular sugar almost never surpasses 30 per cent of the total blood sugar, but is usually far below this. In the presence of increased blood sugar, the corpuscular fraction is relatively decreased, probably on account of a limited sugar capacity of the cells. The corpuscular sugar concentration after the addition of dextrose to the blood is in general smaller at an acid reaction than at a neutral reaction. This is mainly due to an increased volume of the cell at an acid reaction. This increase in volume is due to an increased water content without a corresponding increase of the sugar content of the cell. The volume of the cells has therefore to be considered in the calculation of the cellular sugar fraction.

WILHELM C. HUEPER.

THE IODINE CONTENT OF THE CEREBROSPINAL FLUID. A. HAHN and A. SCHÜRMEYER, *Klin. Wchnschr.* **11**:421, 1932.

Iodine is a normal constituent of the cerebrospinal fluid. With an iodine value of the blood of about 10.62 per cent, there is a concentration in the spinal fluid of about 7.42 per cent—approximately a 70 per cent relation to the iodine content of the blood. With potassium iodide therapy and a hundred-fold increase of the iodine content of the blood, there is only a sixfold increase in the cerebrospinal fluid and the relation to the content in the blood drops to about 3.3 per cent. The permeability of the meninges for iodine increases with fever, and the relation thereby rises to about 7.5 per cent.

EDWIN F. HIRSCH.

WAVELENGTH OF X-RAYS AND BIOLOGIC EFFECT. CARL HOFFMANN, *Strahlentherapie* **43**:140, 1932.

*Opalina ranarum* inoculated into a weakly alkaline culture liquid was exposed to 500 roentgens and 1,000 roentgens and then stained with trypan blue or light green. After a primary state of increased motility following irradiation in the series treated with 500 roentgens, a gradual decrease and ultimate arrest of this activity occurred after three hours. Eight hours later, 50 per cent of the organisms contained dye granules. About 80 per cent of these organisms contained granules of rather coarse character, while the others had very fine granules as seen under normal vital-staining conditions. Exposure to intense light could still elicit movement of the cilia. After eighteen hours, all cells contained coarse dye granules, and some had a diffuse plasmatic stain. The nuclei became pyknotic and intensely stained. The cells were swollen. After two days, some of the organisms showed signs of recovery, but after another twenty-four hours 80 per cent of the opalinae were dead, and the rest showed evidence of degeneration with numerous large stained flakes in the cytoplasm. After ninety-six hours, these organisms had completely recovered and had even increased in number. Attempts to determine the hydrogen ion concentration of the cells with neutral red and methyl red gave inconclusive results. Irradiation with 1,000 roentgens produced similar but more acute changes. After eight hours, 75 per cent of the organisms were dead or fragmented. Irradiation of the culture medium before inoculation was ineffective.

WILHELM C. HUEPER.

## Microbiology and Parasitology

RHINOSPORIDIUM SEEBERI IN A NASAL POLYP: THE FOURTH NORTH AMERICAN CASE. G. S. GRAHAM, *Am. J. Clin. Path.* **2**:73, 1932.

A case is reported of rhinosporidial infection in a nasal polyp. The patient was a 12 year old Negro, born in Georgia, and had resided since the age of 4 in a small

coal-mining village in Alabama. The source of the infection is unknown, no other similar condition having been observed in the community for at least ten years. The case illustrates the remarkable discontinuity of the infection, of which more than forty cases have been reported during the past thirty years from three widely separated localities—India, Argentina and the United States. A preoperative diagnosis should be made without difficulty through recognition of the characteristic spores in the nasal secretions.

AUTHOR'S SUMMARY.

HYMENOLEPIS NANA. T. TSUCHIYA and E. H. ROHLFING, Am. J. Dis. Child. **43**: 865, 1932.

Two cases occurring in children in St. Louis, in addition to the cases previously reported by Goldman, are described. Neither clinical nor laboratory findings were indicative of pathologic conditions in these children. With a view to determining the possible source of the infection, a cross-infection experiment with ova from human sources was carried out in rats. Two of six clean rats were successfully infected. Subsequent experiments showed that five of ten clean rats were infected with the ova of the two rats previously infected with human materials. Since there is the possibility of adapting the human parasites to rats, rats in turn may become one of the sources of the infection in human beings.

AUTHORS' SUMMARY.

ANCYLOSTOMA BRAZILIENSE AND THE ETIOLOGY OF CREEPING 'ERUPTION. WALTER E. DOVE, Am. J. Hyg. **15**:664, 1932.

An incidental intestinal infection of *A. caninum* in a boy in Texas is reported. Penetrations of human skin with mixed larvae of *A. caninum* and *Necator americanus* cultured from a boy produced swelling and short vesicular lesions during the first three days. Penetrations of the skin of a volunteer by larvae of a boy-to-cat strain of *A. braziliense* produced lesions of creeping eruption, which were active for more than thirty-five and fifty-four days. Penetrations of the human skin with a boy-to-dog strain of *A. caninum* produced short linear lesions, which disappeared in less than fourteen days.

AUTHOR'S SUMMARY.

HUMAN AMOEBIC ULCERS. KENTARO HIYEDA and MICHISABURO SUZUKI, Am. J. Hyg. **15**:809, 1932.

When amebas enter the lumen of the large intestine, the intestinal epithelium produces an enormous amount of mucus, which prevents many of the amebas from entering the tissues. The struggle between them and the mucus may last for from two to eight days; this may be verified experimentally. If the amebas succeed in coming in contact with the epithelium, they give rise to necrosis or necrobiosis of the superficial epithelial layer. They multiply when they come in direct contact with the tissue, even though they are actually in the lumen of the intestine. Toxic substances produced by them lead to necrosis of the mucous membrane. In this way, the roughness of erosion of the superficial mucous membrane occurs. The amebas make their way deeper into the intestinal wall, multiply there and produce a large area of necrosis; finally a typical large crater ulcer develops. If the secondary bacterial infection is severe, there results an abscess. A crater ulcer may also result from the bursting of such an abscess, although this is not usual. The amebas within the tissue of the submucosa make their way mostly longitudinally to the axis of the intestine. This is why the ulcers are wider below the surface, and why they build the tunnel-like communications under the intact epithelial covering. If the regenerating activities of the epithelial cells and other intestinal tissues overcome the invading activities of the amebas, the reparation of the ulcer occurs, causing a thickening of submucosa. But *restitutio in integrum* is never reached as long as the amebas are present. If small ulcers occur with only a few amebas in their vicinity, the balance between amebic vitality and tissue

resistance is well kept, and no marked intestinal symptoms may occur. This is the condition of the ameba carrier. Cellular infiltration in amebic ulcers mainly depends on a mixed infection of bacterial origin. None of the leukocytes have a special affinity for the amebas or for their products. The amebas in equilibrium with the local tissues will remain so for long periods, but if the equilibrium is broken, the amebas may become active and give rise to an exacerbation.

FROM AUTHORS' SUMMARY.

INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS ("PROTOZOAN-LIKE BODIES") IN THE SALIVARY GLANDS AND OTHER ORGANS OF INFANTS. S. FARBER and S. B. WOLBACH, *Am. J. Path.* 8:123, 1932.

In the submaxillary glands removed in a series of 183 postmortem examinations on infants, large cells containing intranuclear and cytoplasmic inclusion bodies ("protozoan-like bodies") were found in 22 cases (12 per cent). In addition, there are reported the cases of 2 older children with inclusions in the parotid and submaxillary glands, and 2 instances in which inclusions were found in epithelium-lined spaces of the liver, lungs, kidneys, pancreas and thyroid gland, making a total of 26 new cases to be added to the 25 already recorded in the literature. All the patients in this series were less than 17 months of age, the majority being under 1 year. The inclusions are apparently identical with those found in the submaxillary glands of guinea-pigs, and are generally similar to inclusions found in diseases due to filtrable viruses. Clinical and pathologic studies revealed no association with any distinctive feature or group of symptoms or morbid changes. The frequency of the observation in our postmortem examinations suggests geographic factors affecting occurrence and leads naturally to a suspicion of the existence of an infantile disease caused by a filtrable virus. If there is such a disease, there are no distinctive clinical or pathologic features permitting its recognition in the wards or in the pathologic laboratory. The clinical and pathologic findings in the "positive" instances resemble, in general, those in the entire group studied.

AUTHORS' SUMMARY.

YELLOW FEVER ENCEPHALITIS OF THE MONKEY. E. W. GOODPASTURE, *Am. J. Path.* 8:137, 1932.

A histologic and cytologic study has been made of an encephalitis of monkeys (*Macacus rhesus*) inoculated intracerebrally with the mouse strain of the virus of yellow fever. The lesion is an acute, disseminated encephalomyelitis, extending apparently throughout the central nervous system, affecting the cellular tissues and causing necrosis of ganglion cells, both sensory and motor. Intranuclear inclusions sometimes resembling, but more often differing from, those characteristic of yellow fever have been demonstrated in ganglion cells. On immunologic and histologic grounds it is judged that the virus of the mouse and monkey encephalitis represents a biologically modified strain of the virus of yellow fever. Cytologically, the evidence of morphologically characteristic yellow fever intranuclear inclusions in the brains of encephalitic monkeys inoculated with the mouse virus is inconclusive.

AUTHOR'S SUMMARY.

INTRANUCLEAR INCLUSIONS IN MONKEYS UNACCOMPANIED BY SPECIFIC SIGNS OF DISEASE. W. P. COVELL, *Am. J. Path.* 8:151, 1932.

From these observations and those of Stewart and Rhoads, it seems likely that monkeys must be listed with man, guinea-pigs, rats, rabbits and dogs as animals in which sometimes lurk one or more viruses capable of producing intranuclear inclusions in the absence of recognizable clinical symptoms. Particularly is this of interest when monkeys are employed for experiments with viruses that in the proper environments are definitely disease-provoking, such as those of poliomyelitis, yellow fever, chickenpox and measles.

AUTHOR'S SUMMARY.

TORULA INFECTION. J. W. WATTS, *Am. J. Path.* 8:167, 1932.

Two cases of torula infection are presented. In the first, the infection was generalized, but the symptoms were almost entirely cerebral. A remarkable collection of pathologic changes was present in the brain: diffuse meningitis, granulomas in the meninges, marked endarteritis and proliferation of adventitial elements of the meningeal vessels, an infarct in the pons, areas of softening and focal disappearance of the granular and Purkinje cells in the cerebellum, diffuse changes in ganglion cells, increase of neuroglia, nerve fiber destruction, myelin sheath damage and encephalitis by extension in the striate body. The second case falls into the group which Freeman considers embolic phenomena. Mucicarmin was found to be an excellent differential stain, not only making it easy to identify the organism by its distinctive color, but bringing out details of structure not hitherto recorded. Two strains of yeastlike bodies were isolated: the one producing no pigment falls into the torula group; the one producing pigment appears to be a chromotorula. The organism was nonpathogenic for guinea-pigs, rats and mice. The respiratory tract is probably the portal of entry in most cases. The infarct in the pons, endarteritis of numerous meningeal vessels with the torula in the intima and softenings in the cerebellum in case 1 add weight to the theory that cystic cavities in the deep white and gray matter are embolic phenomena, and that dissemination is by the blood stream.

AUTHOR'S SUMMARY.

CULTIVATION OF *B. LEPRAE* WITH EXPERIMENTAL LESIONS IN MONKEYS. MALCOLM H. SOULE and EARL B. MCKINLEY, *Am. J. Trop. Med.* 12:1, 1932.

The experiments described included (1) the experimental production of granulomatous lesions suggestive of early lesions of leprosy in two species of monkeys by intradermal inoculation of material from human leprosy, (2) the cultivation of acid-fast bacilli (presumably *B. leprae*) from nodules of human leprosy on several artificial mediums in various gaseous environments, and (3) the experimental production of granulomatous lesions suggestive of early leprosy in two species of monkeys by the intradermal inoculation of cultures of acid-fast bacilli from material from human leprosy, grown on artificial mediums. We believe the experiments indicate a step forward in the fulfilment of Koch's postulates for the causative agent in leprosy.

AUTHORS' SUMMARY.

FAT THERAPY IN EXPERIMENTAL TUBERCULOSIS OF RABBITS. SAMUEL A. LEVINSON, *Am. Rev. Tuberc.* 23: 527, 1931.

Feeding egg yolk and cholesterol in oil to rabbits gives rise to a relatively rapid increase of the cholesterol level of the blood. Feeding of cotton-seed oil is not injurious to the mucosa of the gastro-intestinal tract. Animals which were inoculated with tubercle bacilli and which received various fat feedings showed a gain in weight. Animals fed with oil and not inoculated with tubercle bacilli usually lost weight. Overfeeding with cholesterol, leading to oversaturation of the body of the rabbit with cholesterol, does not protect the animals against tuberculosis.

H. J. CORPER.

CALCIFICATION FOLLOWING MASSIVE DOSES OF VIOSTEROL IN EXPERIMENTAL BOVINE TUBERCULOSIS OF GUINEA-PIGS. T. T. WALKER and T. D. SPIES, *Am. Rev. Tuberc.* 24:65, 1931.

Administration of repeated massive doses of viosterol to guinea-pigs with acute bovine tuberculosis causes extensive calcification within the caseous lesions. This calcification is much more marked than that which occurs in similar lesions of tuberculosis without the use of viosterol. Calcium is deposited also in apparently normal tissue of the heart, liver and kidneys of the tuberculous animals treated with viosterol.

H. J. CORPER.

THE DISEASE CAUSED BY FILTRATES OF TUBERCLE BACILLUS CULTURES. M. PINNER and M. VOLDRICH, *Am. Rev. Tuberc.* **24**: 73, 1931.

Filtrable forms of the tubercle bacillus are not demonstrable in pure cultures of the bacillus by the methods tested by Pinner and Voldrich, and they contend that the existence of filtrable forms has not been proved. Filtrates from broth cultures of the tubercle bacillus cause a characteristic disease in guinea-pigs. The nosogenic agent of this disease is not a living virus, because (1) it is not present in whole cultures of the tubercle bacillus grown on solid mediums, (2) it is thermostable, and (3) it is not transmissible by animal passage. This disease is probably caused by a specific tuberculotoxic substance, which may be identical with tuberculin.

H. J. CORPER.

THE LYSIS OF TUBERCLE BACILLI IN SPUTUM. L. ROSENTHAL and V. B. DOLGOPOL, *Am. Rev. Tuberc.* **24**:351, 1931.

Tubercle bacilli undergo lytic changes in sputum if the specimen is allowed to stand a few days before examination. A certain degree of lysis may occur at room temperature. At 37 C., the lysis becomes evident after ten days, following an initial increase in the number of the tubercle bacilli. The optimum temperature for the lysis is from 48 to 60 C. The concomitant flora of the sputum does not affect the staining ability of the tubercle bacilli. The lytic agent is probably the product of autolysis of leukocytes.

H. J. CORPER.

AN ATTEMPT TO GROW PARAMECIA IN PURE CULTURES OF TUBERCLE BACILLI. A. P. DAMEROW, *Am. Rev. Tuberc.* **24**:363, 1931.

Cultures of avirulent human tubercle bacilli will not support growth and reproduction of *Paramecium caudatum*. Attempts to develop tolerance for tubercle bacilli in paramecia were unsuccessful. Tubercle bacilli are rapidly washed from paramecia after they have been in contact for varying intervals of time up to two weeks, indicating that probably the tubercle bacilli were not taken up by the paramecia.

H. J. CORPER.

THE EFFECT OF IRRADIATED ERGOSTEROL ON CALCIFICATION IN PULMONARY TUBERCULOSIS. J. KAMINSKY and D. L. DAVIDSON, *Am. Rev. Tuberc.* **24**: 483, 1931.

Oral administration of small doses of viosterol caused augmentation of serum calcium in patients with pulmonary tuberculosis, but such an increase in serum calcium did not influence to any appreciable extent the degree of calcification in pulmonary lesions, so far as this could be demonstrated by roentgenograms.

H. J. CORPER.

EFFECT OF ENVIRONMENT ON NORMAL AND TUBERCULOUS GUINEA-PIGS. MYRTLE GREENFIELD, *Am. Rev. Tuberc.* **24**:695, 1931.

Normal guinea-pigs live longer in an open building than in either a wooden or a glass building. Guinea-pigs are more able to combat tuberculous infection when housed in an open building than when housed in either a wooden or a glass building. Offspring of tuberculous guinea-pigs housed in an open building are able to live and produce young, while offspring of tuberculous guinea-pigs housed in either a wooden or a glass building live only a short time.

H. J. CORPER.

CHEMICAL AND BIOLOGICAL PROPERTIES OF THE PHOSPHATIDE FROM THE TUBERCLE BACILLUS. C. H. BOISSEvain and C. T. RYDER, *Am. Rev. Tuberc.* **24**:751, 1931.

Intraperitoneal injection into guinea-pigs of a phosphatide (phosphatide A3 of Anderson) causes formation of tuberculous tissue and cutaneous hypersensitiveness.

It is impossible to free this phosphatide from acid-fast bacilli and bacillary débris by centrifugation, precipitation or filtration of its ethereal solution. Filter candles that retain bacilli suspended in water let the same bacilli pass through when suspended in ether. It is possible to free the phosphatide from bacteria and bacterial débris by dialysis of the ethereal solution through a rubber membrane, subsequent solution in a mixture of water, alcohol and ether, and filtration through a Mandler filter candle. Intraperitoneal injection of the purified phosphatide does not cause formation of tuberculous tissue or cutaneous hypersensitiveness. The tissue reaction to phthioic acid is of the nature of a foreign body reaction.

H. J. CORPER.

ACUTE BACTERIAL ENDOCARDITIS DUE TO *BACTERIUM ACIDI-LACTICI*. LEWIS DICKAR, Arch. Int. Med. **49**:788, 1932.

Two cases of endocarditis caused by *B. acidi-lactici*, closely resembling cases of infection with *B. coli*, are reported because of their rarity. More complete bacteriologic studies of cases of sepsis due to *B. coli* will undoubtedly bring to light more examples of infection with *B. acidi-lactici*. The coincidence of involvement of the aortic valve in two cases of endocarditis caused by *B. acidi-lactici* and in two cases caused by *B. coli* is pointed out.

AUTHOR'S SUMMARY.

EXPERIMENTAL YAWS. T. B. TURNER and J. H. CHAMBERS, Bull. Johns Hopkins Hosp. **50**:253, 1932.

The virus of yaws taken early in the disease from ten native Haitians was transferred to both rabbits and monkeys. Eight strains of yaws spirochetes were recovered by the inoculation of rabbits and were propagated through a second generation of this species. Of five monkeys (*Macacus rhesus*) inoculated with material from as many different patients, some presented lesions, but *Treponema pertenue* could not be demonstrated, and there was no assurance, therefore, that the infection had been transmitted to these animals. Rabbits inoculated by three methods—testicular injection, intracutaneous injection and granulating wound—presented definite lesions of yaws only after testicular inoculation. Of thirty-four rabbits inoculated in one or both testes, fourteen presented definite lesions of yaws. A strain of spirochetes of syphilis was successfully transferred from a native Haitian to rabbits. For the transfer of yaws from man to animals, the rabbit proved to be more suitable than the monkey (*Macacus rhesus*). Only testicular inoculation of the rabbit yielded satisfactory results.

ETIOLOGY OF MULTIPLE SCLEROSIS. A. WEIL, J. A. M. A. **97**:1587, 1931.

Repetition of the experiments of Chevassut and Purves-Stewart failed to produce convincing evidence that, in multiple sclerosis, cultures from spinal fluids yield a filtrable virus and that this virus is responsible for the production of the disease.

The fact that spheres and colonies of spheres may more readily be seen in agar cultures of spinal fluids that have given a positive globulin reaction suggests the precipitation of colloidal protein (or lipid) particles, which become visible in the dark field.

AUTHOR'S SUMMARY.

INFESTATION WITH *DIPHYLLOBOTHRIUM LATUM* ON THE EASTERN SEABOARD. M. PLOTZ, J. A. M. A. **98**:312, 1932.

Twenty-one cases of infestation with *Diphyllbothrium latum* are reported from New York City. These are the first from New York State reported in the literature, and include five in which the patients were born in the United States, bringing the total number of reported native cases to thirty-one.

All but two of the patients were females, and all but two Jews.

FROM AUTHOR'S SUMMARY.



ULCERATIVE COLITIS DUE TO CHRONIC INFECTION WITH FLEXNER-Y BACILLUS.  
THOMAS T. MACKIE, J. A. M. A. **98**:1706, 1932.

The Flexner-Y group of dysentery bacilli may cause sporadic cases of mild diarrhea and dysentery. The occurrence of these cases does not require the presence of epidemic conditions. A significant number of the persons concerned may become persistent carriers and may develop ulcerative lesions of the lower part of the bowel, even though the initial acute attack may have been mild. The lesions of chronic bacillary dysentery differ in no respect from those encountered in many of the cases of so-called chronic ulcerative colitis. Intermittence of the excretion of the bacilli is one of the characteristics of chronic infection with the Flexner bacillus, and repeated cultures over a period of weeks may be necessary to demonstrate them. If cultures are taken directly from the floor of the ulcers through the sigmoidoscope, the probability of recovering the organisms is materially increased.

AUTHOR'S SUMMARY.

THE STREPTOCOCCI OF MILK-BORNE SEPTIC SORE THROAT AND SCARLET FEVER. A. W. WILLIAMS and C. R. GURLEY, J. Bact. **23**:241, 1932.

We conclude from our studies on this subject that milk-borne epidemics of septic sore throat and of scarlet fever may be caused, respectively, by one or several agglutinative types of hemolytic streptococci. When scarlet fever predominates, usually the epidemic strain belongs to one of the common scarlet fever agglutinative types; when septic sore throat predominates, the epidemic strain may be one of several agglutinative types, two of which have been demonstrated in these studies and have been called tentatively epidemicus agglutinative type I and epidemicus agglutinative type II.

AUTHORS' SUMMARY.

SPECIFIC ENZYME IN CULTURES OF THE BACILLUS DECOMPOSING THE CAPSULAR POLYSACCHARIDE OF TYPE III PNEUMOCOCCUS. R. DUBOS, J. Exper. Med. **55**:377, 1932.

An improved method is described for the preparation, concentration and purification of the bacterial enzyme that is capable of decomposing the capsular polysaccharide of the type III pneumococcus. The cultural conditions for the growth of the specific micro-organism must be such that the capsular polysaccharide is completely decomposed before any appreciable amount of free enzyme is released into the medium. This reduces to a minimum the decomposition of the specific substrate by the free enzyme. As a result, a larger part of the specific substance remains as a source of energy for the growing micro-organism, and less enzyme is lost through inactivation during the course of decomposition of the specific substrate. A marked stimulation of growth and of the production of the enzyme occurs when small amounts of yeast extract are added to the medium, and when the cultures are incubated under conditions of increased aeration. Special emphasis is placed on the fact that, thus far, appreciable amounts of the specific enzyme have been obtained only when the capsular polysaccharide itself, or the aldobionic acid derived from it, was present in the culture medium.

AUTHOR'S SUMMARY.

THE ACTION OF A SPECIFIC ENZYME UPON THE DERMAL INFECTION OF RABBITS WITH TYPE III PNEUMOCOCCUS. K. GOODNER, R. DUBOS and O. T. AVERY, J. Exper. Med. **55**:393, 1932.

The action of the enzyme that specifically decomposes the capsular polysaccharide of the type III pneumococcus has been tested in dermal infections of rabbits with this pneumococcus. When injected in sufficient amounts, the enzyme is capable of bringing about a favorable and early termination of the experimental disease, which ordinarily is fatal in nearly all instances. The results of the present study yield further evidence that the capsular substance is of great importance

in pneumococcic infection, since, so far as known, the only action of which the specific enzyme is capable is that of decomposing the capsular polysaccharide.

AUTHORS' SUMMARY.

FILTRABILITY OF TUBERCULOUS VIRUS. C. CALLERIO, *Ztschr. f. Tuberk.* **63**:198, 1932.

Ninni reported that injection of filtrates from cultures of the tubercle bacillus into lymph nodes permitted demonstration of acid-fast elements in the nodes in a high percentage of tests. He made the injections into the tracheobronchial lymph nodes in guinea-pigs. Callerio repeated these experiments, following Ninni's technic in every detail and using even the same strain of tubercle bacilli. Seventy-two guinea-pigs were used, but the results in all cases were negative.

MAX PINNER.

DEMONSTRATION OF SMALL NUMBERS OF TUBERCLE BACILLI. E. DE CARVALHO, *Ztschr. f. Tuberk.* **63**:305, 1931.

Tubercle bacilli cannot be found microscopically unless from 10,000 to 100,000 are present in 1 cc. of the material. By cultural methods and by inoculation in animals they can be demonstrated when only from 1 to 10 are contained in 1 cc. Using artificial mixtures of negative sputum and a known amount of bacilli, Carvalho found that cultural methods are about as good as inoculation in animals. The treatment of sputum with acids or with alkali will at times prevent small numbers of bacilli from growing on mediums and from producing tuberculosis in guinea-pigs. The most sensitive method for the demonstration of tubercle bacilli is that of the injection of untreated material into guinea-pigs. MAX PINNER.

ROBERT KOCH-HEFT, *Ztschr. f. Tuberk.* **64**:1, 1932.

This issue is dedicated to commemoration of the fiftieth anniversary of the discovery of the tubercle bacillus by Robert Koch. The single articles deal with certain phases in the development of knowledge of tuberculosis since 1882 and represent reviews rather than original contributions. As such, they are not well suited for abstracting, but since several of them are interesting summaries, a list of the single papers follows:

- E. von Romberg: The Significance of the Discovery of the Tubercle Bacillus for Clinical Work.
- R. Sauerbruch: Robert Koch.
- R. Philip: The Discovery of the Tubercle Bacillus: Some Consequences.
- P. Huebschmann: What Did the Discovery of the Tubercle Bacillus Mean for Pathologic Anatomy?
- L. Bernard: The Demonstration of Koch's Bacillus in Sputum and the Diagnosis of Pulmonary Tuberculosis.
- B. Lange: The Work of Robert Koch and the Newer Research in Experimental Tuberculosis.
- A. Calmette: Koch's Bacillus and the Invisible Elements of the Tuberculosis Virus.
- E. R. Baldwin: The Cultivation of a Human Tubercle Bacillus During a Period of Forty-One Years.
- W. von Drigalski: The Significance of the Discovery of the Tubercle Bacillus for Social Hygiene.
- B. Möllers: The Decrease in the Mortality from Tuberculosis During the Last Fifty Years.
- O. Roepke: The Diagnostic and Therapeutic Significance of Tuberculin.
- E. Küster and W. Stempel: The Standardization of Tuberculin by Intracutaneous Tests in Tuberculous Guinea-Pigs.
- E. R. Long: The Chemistry of the Tubercle Bacillus.

- B. Bang: The Significance of the Discovery of the Tubercle Bacillus for the Eradication of Tuberculosis in Domesticated Animals.  
 H. Meissner: The Campaign Against Bovine Tuberculosis.  
 A. St. Griffith: The Types of Tubercle Bacilli in Lupus.  
 E. Löwenstein: Remarks About Tubercle Bacillus Bacillemia.

MAX PINNER.

### Immunology

THE CRAIG COMPLEMENT-FIXATION TEST FOR AMEBIASIS IN CHRONIC ULCERATIVE COLITIS. EVERETT D. KIEFER, *Am. J. M. Sc.* **183**:624, 1932.

It is extremely difficult to differentiate between chronic ulcerative colitis and chronic amebic colitis. When the Craig complement-fixation test for infection with *Endamoeba histolytica* was applied to nineteen patients with chronic ulcerative colitis fifteen gave a strongly positive reaction. No conclusions as to the significance of this are drawn, but it is suggested that chronic ulcerative colitis may be a pyogenic infection superimposed on amebic ulceration. Abstracts of the histories of the nineteen patients are reported.

AUTHOR'S SUMMARY.

DESENSITIZATION OF TUBERCULOUS GUINEA PIGS. JOHN WEINZIRL and RUBY M. BOHART, *Am. Rev. Tuberc.* **23**:393, 1931.

Weinzirl and Bohart made tests to determine the value, for purposes of desensitization, of (a) the dialyzed filtrate of a culture of the tubercle bacillus on Long's medium, (b) tuberculoproteins derived from the filtrate of the culture on Long's medium, (c) tubercle bacilli killed by carbolfuchsin and (d) tubercle bacilli killed and treated with ether. Desensitization, as indicated by the intradermal test, is only partially successful, but the reaction is frequently reduced below 4 plus. If, however, the systemic reaction is considered, desensitization seems to be more complete. Desensitization largely eliminates prostration following the administration of tuberculo-antigens. Of the antigens tested, the ether-killed and treated tubercle bacilli seemed to give the most satisfactory results. The size of the dose must vary with the antigen employed. It should be kept sufficiently low to avoid a violent systemic reaction. The doses should be spaced so as to avoid (a) coalescing of the doses and (b) a return of sensitization. An interval of less than five or more than ten days between doses is undesirable.

H. J. CORPER.

COMPLEMENT FIXATION WITH URINE IN TUBERCULOSIS. M. E. PARKER, *Am. Rev. Tuberc.* **23**:733, 1931.

Four hundred and eighty-three complement-fixation tests for the detection of tuberculosis were made on urines of tuberculous and nontuberculous persons. It was found that the presence of specific antigens or antibodies in the urine, as demonstrated by complement fixation, is highly indicative of a tuberculous lesion somewhere in the body, but not necessarily in the urinary system. A negative result has no diagnostic significance.

H. J. CORPER.

THE PHAGOCYTOSIS OF TUBERCLE BACILLI. M. BORQUIST and C. ROWE, *Am. Rev. Tuberc.* **24**:172, 1931.

Micromanipulation of monocytes and clasmotocytes shows that they differ in consistency, a monocyte being fragile and easily disintegrated, and a clasmotocyte being distinctly more resistant, more sticky and more elastic than a monocyte. These cells with ingested tubercle bacilli were studied (1) on flat preparations both with neutral red and without stain for periods of from two to eleven hours, and (2) on fixed and stained smears of mixtures of cells and bacteria that had been incubated in hanging drops. Monocytes, clasmotocytes and epithelioid cells with ingested bacilli were studied in smears of pulmonary tissue and peritoneal

exudates obtained from tuberculous animals. There is no evidence that ingested tubercle bacilli of bovine strains are fragmented by any of the cells studied. Bacilli inside cells neither took up neutral red stain nor appeared disintegrated. Epithelioid cells contained many more bacilli than other cells, but no greater number of life cycle changes were seen within them. These experiments suggest that the longevity of the epithelioid cell, rather than a multiplication of bacilli within it, may account for its greater content of bacilli as compared with the clasmatocyte.

H. J. CORPER.

EFFECT OF ACID HYDROLYSIS OF THE POLYSACCHARIDE FROM THE HUMAN TUBERCLE BACILLUS. K. L. McALPINE and P. MASUCCI, *Am. Rev. Tuberc.* **24**:729, 1931.

The polysaccharide obtained from the filtrate of a culture of the human tubercle bacillus on Long's synthetic medium has been split by mild acid hydrolysis into two fractions: an alcohol-insoluble fraction, containing large amounts of combined mannose and a small amount of free reducing sugars, which is readily obtained in the form of a white, dry powder having a relatively high optical rotation and a low precipitin titer; and an alcohol-soluble fraction, containing most of the arabinose and large amounts of free reducing sugars, which is a bright yellow gum of low optical rotation and relatively high precipitin titer. During acid hydrolysis of the polysaccharide the lowering of the precipitin titer proceeds at a much greater rate than the cleavage of the polysaccharide into the simple sugars.

H. J. CORPER.

POLYSACCHARIDE IN FILTRATES OF CULTURES OF THE TUBERCLE BACILLUS ON LONG'S SYNTHETIC MEDIUM. P. MASUCCI, K. L. McALPINE and J. T. GLENN, *Am. Rev. Tuberc.* **24**:737, 1931.

The growth of human and bovine tubercle bacilli on Long's synthetic medium is characterized by the appearance in the medium of substances that form a precipitate with serum prepared against human tubercle bacilli. The filtrate of the bovine culture contains much less of the reacting substance (polysaccharide) than that of the human culture. In the polysaccharide, the active radical appears to be the pentose.

H. J. CORPER.

LYSOZYME AND TUBERCULOSIS. H. J. CORPER, *Am. Rev. Tuberc.* **25**:59, 1932.

Lysozyme prepared from the liver or the spleen of the dog or the guinea-pig did not have an inhibitory or bactericidal effect on tubercle bacilli in vitro. Its action does not appear to be a factor in organic susceptibility to tuberculosis in these animals. The experiments reported also make it appear unlikely that lysozyme plays a significant part in susceptibility or resistance to tuberculosis in man.

H. J. CORPER.

SEROLOGICAL REACTIONS WITH HEMOLYTIC STREPTOCOCCI IN ACUTE BACTERIAL INFECTIONS. W. S. TILLET and T. J. ABERNETHY, *Bull. Johns Hopkins Hosp.* **50**:270, 1932.

Serums from patients acutely ill with a variety of bacterial infections are capable of agglutinating certain strains of hemolytic streptococci. This capacity is demonstrable early after the onset of the illness, persists during the active stage, and disappears soon after recovery. Four strains of *S. hemolyticus*, derived from different diseases, are agglutinable. Agglutinability is destroyed by heating cultures to the thermal death point. Agglutinable cultures killed with formaldehyde are not altered. The protein fraction of serum precipitated by carbon dioxide, under the conditions described, contains the constituent reactive with streptococci. By this method, serums from patients with typhoid fever were fractionated, and the

agglutinins for typhoid bacilli and hemolytic streptococci were separated. The implications of the parallelism of altered suspension stability of red blood cells and streptococcic agglutination are discussed.

AUTHORS' SUMMARY.

CARBOHYDRATES ADSORBED ON COLLOIDS AS ANTIGENS. J. ZOZAYA, J. Exper. Med. **55**:325, 1932.

Evidence is given that polysaccharides can be rendered antigenic by haptogenic adsorption on a colloid carrier. The polysaccharides studied were those of *Bacillus anthracis*, *Meningococcus*, *Streptococcus viridans* (Bargen), *Bacillus proteus*, *Bacillus morgani*, *Bacillus dysenteriae*, both the Shiga and the Hiss type, and *Pneumococcus*. With the polysaccharide of type III pneumococci, I have been unable in six weeks to produce any detectable protective antibodies, but was able to produce anticarbohydrate antibodies. All the bacterial carbohydrates were nonantigenic alone when used in the doses indicated, though containing some nitrogen. Dextran, which was free from nitrogen, was also rendered antigenic by the adsorption method.

AUTHOR'S SUMMARY.

IMMUNOLOGICAL REACTIONS BETWEEN DEXTRAN POLYSACCHARIDE AND SOME BACTERIAL ANTISERA. J. ZOZAYA, J. Exper. Med. **55**:353, 1932.

Dextran, the synthetic polysaccharide produced by *Leuconostoc mesenteroides* from saccharose, reacts immunologically with antipneumococcus serums and with serums prepared against some of the *Salmonella* and some of the types of *Streptococcus viridans* (Bargen). This immunologic relationship is independent of the specific antipolysaccharide antibodies of these serums, suggesting the existence of a distinct antibody produced by an active group of the specific bacterial polysaccharide, which is similar to or identical with the active group of the dextran polysaccharide.

AUTHOR'S SUMMARY.

THE MECHANISM OF PNEUMOCOCCUS IMMUNITY. H. K. WARD, J. Exper. Med. **55**:511 and 519, 1932.

Type III antipneumococcus serum, after absorption with the specific carbohydrate, no longer forms a precipitate with the carbohydrate, but still has a definite, although diminished, bactericidal action on virulent pneumococci. Such an absorbed antiserum still retains some of its power to neutralize the antibactericidal effect of the specific carbohydrate in a bactericidal test, showing that absorption with the carbohydrate does not remove all the anticarbohydrate antibody from an antiserum. This carbohydrate neutralization test is a much more delicate method for detecting the anticarbohydrate antibody (precipitin) than the precipitin test. There is, therefore, no necessity to predicate another antibody to explain the bactericidal action of a carbohydrate-absorbed antiserum or a similar result in a mouse protection test. The specific carbohydrate has a definite antibactericidal action, but it is demonstrated that, were it present in this form in the body during pneumonia, it could not conceivably be produced in sufficient quantity to influence the disease.

There is in the filtrate of a five day broth culture of type III pneumococcus a type-specific substance which has a very powerful antibactericidal action. If the precipitinogen content of the broth filtrate and the specific carbohydrate are taken as the basis of comparison, it requires approximately one thousand times as much antiserum to neutralize the broth filtrate as is necessary to neutralize the specific carbohydrate. The active substance in the broth filtrate appears to be related to the specific carbohydrate. Its possible nature is discussed. A similar substance, but in stronger concentration, was found in the filtrate of a lung obtained at autopsy in a case of type III pneumonia. The influence of this substance on the disease is discussed. One specimen of type III convalescent blood, though comparatively weak in anticarbohydrate antibody (precipitin) was better

able to neutralize the broth filtrate and the lung filtrate than a corresponding mixture of normal blood and antiserum. Two specimens of type II convalescent blood neutralized the type III broth filtrate efficiently.

AUTHOR'S SUMMARIES.

THE RÔLE OF CONDITIONAL REFLEXES IN IMMUNITY. J. METALNIKOW, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:338, 1932.

This article is a criticism of the results published by E. Friedberger and I. Gurwitz (*Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:173, 1931; abstr., *ARCH. PATH.* **13**:518, 1932)—results that entirely contradicted those originally published by Metalnikow (*Ann. Inst. Pasteur.* **46**:131, 1931; abstr., *ARCH. PATH.* **12**:496, 1931). Friedberger and Gurwitz did not adhere to the principles as laid down by Pavlov and his school for experimental work on conditional reflexes. Their method of evaluating results carried with it a serious source of error. Metalnikow believes that this fully explains the contradictory results.

I. DAVIDSOHN.

ADSORPTION OF THE ANTIGENIC FRACTION FROM ALCOHOLIC ORGAN EXTRACTS, FROM LECITHIN AND CHOLESTEROL. A. J. WEIL and J. BERENDES, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:341, 1932.

The adsorption and subsequent separation from the adsorbent was accomplished with kaolin. Alcoholic tissue extracts of heterophilic and nonheterophilic organs were used. The antigenic fractions of cholesterol, dihydrocholesterol and di-stearyl-lecithin were adsorbed in a similar manner, but subsequent separation from kaolin was not possible.

I. DAVIDSOHN.

THE SO-CALLED FRACTIONS OF THE COMPLEMENT. KARL DEISSLER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:365, 1932.

The so-called fourth fraction of the complement is the one that is destroyed by the action of ammonia and ammonium salts; the third fraction is destroyed by yeast and cobra poison. The two fractions complement each other. The "Mittelstück" and "Endstück" are not affected by the action of the aforementioned substances. The fourth fraction acts directly on the sensitized antigen, which is contrary to the behavior of the third fraction. In the complement fixation the fourth fraction is used up.

I. DAVIDSOHN.

IMMUNIZATION WITH LECITHIN FROM HUMAN BRAIN. F. PLAUT and H. RUDY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:385, 1932.

There was no specific antigenic response in rabbits immunized with a mixture of hog serum and a purified lecithin product, prepared by Plaut and Rudy from human brain. Addition of pure cholesterol to the hog serum-lecithin mixture did not change the result. The antigenic response obtained with the commercial preparations of lecithin is probably due to admixtures, possibly the so-called egg yolk antigen. The results are a confirmation of the report of Levene, Landsteiner and Van Der Scheer (*J. Exper. Med.* **46**:197, 1927).

I. DAVIDSOHN.

THE RELATION OF THE BLOOD ANTIGENS IN *BACILLUS PARATYPHOSUS* B AND *BACILLUS DYSENTERIAE* SHIGA TO CERTAIN ANIMAL CELLS AND TO HUMAN RED BLOOD CELLS. M. EISLER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:392, 1932.

*B. paratyphosus* B produces in rabbits a serum that agglutinates specifically but does not lake human blood A, and agglutinates as well as lakes sheep blood. These antibodies react with a suspension of *B. paratyphosus* B and with the aqueous and alcoholic extracts of this organism, but not with the carbohydrate

fraction. They react also with the alcoholic extracts of sheep blood and guinea-pig kidney. Pure alcohol inhibited the lysis of sheep blood as well as did alcoholic extracts of *B. paratyphosus* B. Human A blood and peptone Witte fix the anti-A agglutinins. *B. paratyphosus* B has only a limited binding capacity. The two types of antibodies in the immune serum are of the Forssman type and are produced by fractions of the Forssman antigen in the bacteria. The complete Forssman antigen is present in the tissues of the guinea-pig. The two partial Forssman antigens in *B. paratyphosus* B are not identical, the human A-fraction being water-soluble and nonalcohol-soluble, while the sheep fraction is identical with the portion of the Forssman antigen present in all human red blood cells. *B. dysenteriae* Shiga produces in the goat an agglutinin for human blood of all types, but no lysin; in the rabbit it produces agglutinins and lysins for sheep blood but none for human blood. The carbohydrate fraction contains both antigens (the human and the sheep). The sheep antibodies in the immune serums are of the Forssman type and are bound by guinea-pig organs and by the bacteria and their carbohydrate fraction, but not by their alcoholic extracts. The Forssman fraction in *B. dysenteriae* Shiga is not identical with the one in human blood. The antihuman agglutinin produced in the goat is heterophilic, but not of the Forssman variety. The two blood antigens are not present in all strains of *B. dysenteriae* Shiga. Immune serums produced by strains possessing these antigenic fractions are specific for such strains and do not react with strains lacking the same antigenic qualities.

I. DAVIDSOHN.

SEROLOGIC DIFFERENTIATION BETWEEN THE ANTERIOR AND THE POSTERIOR LOBE OF THE HYPOPHYSIS. E. WITEBSKY and H. O. BEHRENS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:415, 1932.

Organ-specific antiserums were produced for the posterior but not for the anterior lobe of the hypophysis. They reacted frequently, though not constantly, with brain antiserums and were not species-specific. A very distinct serologic differentiation was obtained between the posterior lobe of the hypophysis and the spinal cord.

I. DAVIDSOHN.

SERODIAGNOSIS OF TUBERCULOSIS. F. KLOPSTOCK, *Ztschr. f. Tuberk.* **63**:81, 1931.

In systematic studies it was found that the most suitable antigen for complement fixation in tuberculosis is the alcohol-soluble fraction of tubercle bacilli the wax of which has been removed. The action of this antigen can be enhanced by the addition of some of the wax. A positive complement fixation does not necessarily signify active tuberculosis.

MAX PINNER.

EXPERIMENTS WITH PULMONARY LIPASES. D. KANOCZ, *Ztschr. f. Tuberk.* **63**:113, 1931.

From normal human lungs a lipase can be isolated according to a process developed by Willstätter-Waldschmidt-Leitz. Tubercle bacilli exposed to this lipase lose their lipoids and are transformed into granules. Such granules immunize guinea-pigs effectively against a massive infection with tubercle bacilli. The lipase is bacteriostatic in vitro.

MAX PINNER.

## Tumors

THE NATURE AND ETIOLOGY OF CANCER. H. GIDEON WELLS, *Am. J. Cancer* (supp.) **15**:1919, 1931.

Wells emphasizes that cancer is not an exclusively human affliction. The idea that cancer is especially common in man is correct, simply because man is one of

the few species of animals that is commonly permitted to reach old age. The study of the disease has served to remove all doubts that cancers in animals and man represent fundamentally the same disease. The differences observed depend either on anatomic peculiarities of different species or on other differences between species, for the frequency of each type of cancer varies with each species. In recent cancer research the trend of results is in support of the view that cancer is usually the result of protracted stimulation of tissue growth by nonspecific agents, acting on tissues the susceptibility of which is determined by their hereditary background. With regard to hereditary influence on susceptibility and resistance to cancer, Wells is of the opinion that the existence of this factor is established both for man and animals.

Is cancer increasing in frequency? Wells says, "If I were to take of the statistics the only ones that seem to me, as a pathologist, likely to be reasonably accurate, namely, the data on external or accessible cancer, corrected for age and sex, I should have to conclude that in all probability cancer is increasing to just about the extent that people are kept from dying of something else." The statement that cancer is rare among "uncivilized" races rests on the "most worthless of evidence."

A scholarly review of the subject, it is condensed to about fifty pages, and is here only partly abstracted.

B. M. FRIED.

THE HISTOGENESIS OF THE SO-CALLED "BASAL-CELL CARCINOMA." SAMUEL R. HAYTHORN, *Am. J. Cancer* (supp.) **15**:1969, 1931.

Of 412 tumors from hair-bearing areas studied for their relationship to hair follicles, 144 were of noncornifying basal cell type. The conception of Krompecher, who traced the genesis of these neoplasms to the basal cells, is denied by Haythorn, who produces evidence that they originate from the hair matrix. Thus evidence of imperfect hair shafts was found in a majority of the new growths. Pigment was present in a few of the tumors, suggesting the distribution of the pigment in the hairs. A heavy basement membrane resembling the vitreous membrane of the hairs was constantly conspicuous about the nests of tumor cells.

On the basis of histologic studies, Haythorn affirms that the tumors should not be called "basal cell" but "hair matrix" cancers. The article is accompanied by thirty-eight good photomicrographs.

B. M. FRIED.

LEUKOPLAKIA OF THE ESOPHAGUS. GEORGE S. SHARP, *Am. J. Cancer* (supp.) **15**:2029, 1931.

Sharp affirms that leukoplakia of the esophagus is common. He is of the opinion that it is frequently associated with esophageal carcinoma.

B. M. FRIED.

CANCER OF THE THYROID: ITS RADIOSENSITIVITY. CUSHMAN D. HAAGENSEN, *Am. J. Cancer* (supp.) **15**:2063, 1931.

Haagensen has grouped cancers of the thyroid gland into five distinct groups—each group having a characteristic natural history and a typical structure. Thirty cancers so classified were studied from the point of view of their reaction to irradiation, such as primary regression and definite cure. The data indicated a moderate radiosensitivity of the less anaplastic types of cancer of the thyroid gland, most marked in the adenocarcinoma group. The most rapidly growing and anaplastic types appeared to be uniformly radioresistant. This finding is not in agreement with hitherto proposed laws of radiosensitivity. In determining the type of treatment, Haagensen emphasizes the histologic type of the tumor, its size and its extent.

B. M. FRIED.



TUMORS OF THE THYMUS. HARRY M. MARGOLIS, Am. J. Cancer (supp.) **15:2106**, 1931.

Tumors of the thymus gland may originate from the parenchyma (the cells of the reticulum, the small thymic cells or thymic lymphocytes, and the corpuscles of Hassall) and from the stroma (lymphosarcomas, fibrosarcomas, myosarcomas or lipomas); since the histogenesis of the thymic elements is still *sub judice*, the classification of thymic neoplasms is not possible at present. Margolis considers that the term thymoma for all tumors derived from the parenchyma of the organ is more acceptable than their discrimination into carcinomas and lymphosarcomas.

Tumors included in the group of lymphosarcomas are probably not other than a morphologic variation of a type cell derived from the thymic reticulum. The designation sarcoma should be reserved for tumors derived from elements of the stroma.

The observation of typical corpuscles of Hassall resulting from degeneration of epithelial cells of tumors apparently derived from the thymic reticulum suggests an analogous mode of formation of these thymic corpuscles in the normal thymus. The article is accompanied by very good photomicrographs and by a complete bibliography.

B. M. FRIED.

PRIMARY MELANOCARCINOMA OF THE GALL BLADDER. SOL ROY ROSENTHAL, Am. J. Cancer (supp.) **15:2288**, 1931.

A case of primary melanocarcinoma of the gallbladder with multiple metastases is reported. By studying the various metastatic nodules, it was found that the melanin was present in the cytoplasm in the early stages as a dust-like, light brown pigment. The latter was usually associated with death of the cell and liberation of the melanin, which was in turn found in the cytoplasm of elongated, mobile cells as pigment granules of various sizes, ranging in color from dark brown to black.

B. M. FRIED.

HEMANGIO-ENDOTHELIOMA OF THE THYROID GLAND. CARL O. RICE, Am. J. Cancer (supp.) **15:2301**, 1931.

Rice reports a case of hemangio-endothelioma of the thyroid gland in a woman of 50, which, he states, is the first to be described in the American literature. The tumor does not vary histologically from tumors of the same type found elsewhere in the body. Roentgen therapy, he believes, may prove of value in the treatment.

B. M. FRIED.

PRIMARY PULMONARY SARCOMA. HOWARD A. BALL, Am. J. Cancer (supp.) **15:2319**, 1931.

Ball discriminates between two types of primary pulmonary sarcoma: (1) pulmonary fibrosarcoma with greater or less differentiation, occurring in adults and having a slowly progressive course and (2) pulmonary round cell sarcoma, occurring in young subjects and exhibiting a more rapid course. The latter probably originates from the mesenchymal cells lining the air sacs. He reports a case of the first variety, which occurs with greater frequency. A review of the literature on the occurrence of the disease since 1900 is given. The article is well illustrated and is accompanied by a detailed bibliography.

B. M. FRIED.

SUPRARENAL NEUROCYTOMA WITH METASTASES. M. G. PETERMAN, Am. J. Dis. Child. **43:655**, 1932.

The tumor reported arose from the left suprarenal gland in a boy, aged 3. There was a retroperitoneal mass on the left, which was dark red, weighed 500 Gm., and contained areas of cystic degeneration. The kidney could easily be separated from the mass. The suprarenal cortex was found intact, the tumor replacing the medulla. One metastasis was found in the sacrum, another in the

petrous portion of the temporal bone and a third in the left hemisphere of the cerebellum. The tumor revealed a variegated architecture, but mostly an infiltration of small, round, sarcoma-like cells, and the diagnosis was neurocytoma. The metastases showed the same picture.

PAUL MERRELL.

FORMALDEHYDE TREATMENT OF A TRANSPLANTABLE RAT CARCINOMA. RAYMOND E. GARDNER and ROSCOE R. HYDE, *Am. J. Hyg.* **15**:509, 1932.

One per cent formaldehyde in physiologic solution of sodium chloride when injected directly into and around Walker rat carcinoma no. 256 produced, after prolonged treatment, complete regressions in a low percentage, but evoked no immunity as tested by reimplantation. Restriction may be attained with certain tumors, but does not seem promising even for experimental investigation. Fresh whole chicken blood and chicken egg albumin, distinctly different proteins antigenically, do not produce inhibitory effects when injected directly into and around a progressive Walker rat carcinoma no. 256.

AUTHORS' SUMMARY.

PRIMARY ANGIOFIBROMA OF THE DIAPHRAGM. E. BURVILL-HOLMES and WILLIAM BRODY, *Am. J. M. Sc.* **183**:679, 1932.

A case of primary angiofibroma of the diaphragm is reported. The tumor lay between the leaflets of the right dome of the diaphragm. It was composed principally of fibrous tissue, which contained many angiomatous areas, some of capillary and some of cavernous type. The patient died of pulmonary tuberculosis, the presence of the tumor not being suspected. The case is of interest because of the rarity of primary tumors of the diaphragm, less than a score of which have been reported.

SANDER COHEN.

CANCER IN THE ANAMITE. J. BABLET, *Hyg. soc.* **60**:1014, 1931.

A careful study of statistics in the important centers in Indo-China resulted in certain conclusions. The Anamese in Cochinchina as in Tonkin appear as susceptible to cancer as inhabitants of temperate countries.

Certain localizations have an abnormal frequency in Indo-China. Carcinoma of the penis and neoplasms of the cervix and mouth are frequent in the Anamese, while cancers of the digestive organs are rarely observed. Histologically, the only interesting point is the elevation in the proportion of tumors of the conjunctiva—one sarcoma to six epitheliomas in place of one to twenty in temperate climates.

The susceptibility of the two sexes does not appear to be different. The cancer age is notably lowered in the Anamese.

These conclusions, which represent a step forward in the knowledge of cancer in Indo-China, will undoubtedly be modified with further organization of cancer centers.

H. S. THATCHER.

PERITHELIAL ENDOTHELIOMA OF THE BULBI PILORUM. H. HAMDI, *Ztschr. f. Krebsforsch.* **32**:377, 1930.

This is a short report of a case of sarcoma of the axilla with cell nests which reproduced with some faithfulness the histologic features of hair follicles, and which the writer terms a perithelial endothelioma of the hair follicles.

EGGERS, Omaha.

EXPERIMENTAL RESEARCHES ON PRECANCEROUS CHANGES IN THE SKIN AND SKIN CANCER. GUSTAV GULDBERG, *Acta path. et microbiol. Scandinav.*, supp. **8**, 1931, p. 223.

The object has been to follow in rabbits and mice the local effects of tar on the skin and its various elements, especially the nerves and vessels, from the first

up to the cancer stage. In mice, the effect of tar is found to last longest on the portion between the shoulder blades. Tar of high distillation fractions produces more distinct dermatitis than the lower fractions and than ordinary gas-works tar, but at the same time it is more toxic. The first phase, the reversible, is the period until malignancy appears, and the cutaneous changes in this period may disappear, if the painting is discontinued. But if malignancy has arisen, the process has passed over to the irreversible phase, with subsequent atypical and infiltrating growth of cells. The maximum of tar irritation does not coincide with the maximum of carcinogenic effect. Sometimes there is only atrophy of the skin after previous pronounced symptoms of inflammation. A general tar poisoning with reduction of the power of resistance is not necessary for the development of papilloma and cancer on the painted area. As early as in the first week, epidermal changes with increasing hyperplasia and hyperkeratosis appear. Most of the hairs become atrophied, but some of the root sheaths become the seat of hyperplasia and cyst formation. Papilloma may develop from the hair follicles or from connective tissue proliferation. The carcinoma forms macroscopically two main types, the papillomatous and the *ulcus rodens*. There are no grounds for regarding incipient carcinoma as a specific form (carcinoid). The carcinoma presents an extreme differentiation of cells and we can distinguish between differentiated and undifferentiated types of cells. In several tar carcinomas, spindle cells were found in varying proportions, and in some of the carcinomas we could follow the transition from squamous epithelium to spindle cells. Some of the tumors were purely spindle-celled. That the cells in such growths are morphologically altered epithelium is possible, but has not been proved. It is also, however, possible that the tumors are pure sarcomas. Pure polymorphous-celled sarcoma has been obtained in the skin of one rabbit after painting with tar. In the corium during the first month of painting there is inflammation of varying intensity. The cell forms vary: in the first two weeks chiefly leukocytes, afterward chiefly lymphocytes, while at the end of the first and in the second month of painting the fibroblasts and fibrillary elements of the corium increase, and at the same time immigration of mast cells occurs in mice, but not in rabbits. Besides the increase in connective tissue there is often a much less pronounced hyperplasia of the elastic tissue. Any absolute parallelism between the increase and the decrease in the elastic tissue and the formation of cancer was not observed. In experiments on mice with heated tar (55 C.) and with tar of high distillation fractions (from 300 to 400 C.), the local inflammatory reaction lasts longer than when gas-works tar of ordinary temperature is used (from 35 to 37 C.). Besides the hyperemia in the painted area there can also be observed in vitally stained preparations a distinct reflex hyperemia. In the nerves of the skin there arises, on painting with tar, both in mice and rabbits, an acute degeneration in the precancerous stage. This degeneration sets in during the first two weeks of painting and is pronounced at the beginning of the second month. Resection of the peripheral nerves leads to a somewhat earlier development of papilloma in the skin on the side on which operation was done than on the control side, but no earlier formation of cancer. Extirpation of the superior sympathetic cervical ganglion together with the adjoining parts of the *nervus sympathicus* leads to an earlier development of papilloma and cancer in the skin on the side on which operation was done than on the control side. The neuroparalytic hyperemia with increased supply of blood and increased transudation probably constitutes a contributory factor to the earlier occurrence of epithelial hyperplasia and cancer. The nerves therefore have a certain significance in the development of epithelial hyperplasia and growths in the skin. Mainly on the basis of the experiments in resection of nerves in the ears of rabbits, the author adopts the view that vascular changes play an essential rôle in the epithelial hyperplasia and formation of papilloma, but as to what part they play in the development of the cancer itself no definite conclusions can be drawn. In the author's opinion, the cancerization of the epithelial cells is due to an alteration of environment, probably caused by circulatory disturbances and processes arising therefrom. The changes in both the corium and the epidermis must be conceived

as the result of a toxic action of the tar. The toxic substance in the tar must be supposed to act directly on the various elements of the corium, especially on nerves and blood vessels. The long-continued application of tar leads to alterations in the functions not merely of one single kind of tissue but of all the tissue elements.'

FROM AUTHOR'S SUMMARY.

### Medicolegal Pathology

IMPORTANCE OF HISTOLOGIC EXAMINATIONS OF LUNGS IN LEGAL MEDICINE.

A. FOERSTER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18:507**, 1932.

The hydrostatic test of live birth has no practical value in cases of advanced putrefaction, since it leads to erroneous interpretations. Microscopic examination of the lungs with hematoxylin-eosin alone is not sufficient to decide positively if the distention of the alveoli occurred on account of penetration of amniotic fluid or if it was due to an intravital inflation with air. Weigert's elastic stain, however, allows a quite reliable conclusion, since in instances of respiration the elastic fibers of the alveoli appear tense and stretched, presenting a round or semicircular arrangement, while in cases of aspiration of fluids the elastic fibers are wavelike in appearance. Cadaveric formation of gas is found mainly in the interstitial tissue of the fetal lung, often in the close vicinity of the blood vessels, and the gas bubbles are never surrounded by elastic fibers.

E. L. MILOSLAVICH.

TRAUMA AND RUPTURE OF THE AORTA. ERNST HAMMER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18:541**, 1932.

A young, healthy sportsman, 25 years of age, suffered suddenly a spontaneous rupture of the ascending aorta, 2 cm. above the aortic leaflets, causing death within eighteen hours. Another healed tear and an old intramural hematoma were found in the aortic arch. Microscopic examination of the aorta in the region of the recent laceration showed many peculiar defects within the elastic apparatus of the media; the elastic fibers appeared torn and pulled apart, but there was no evidence of inflammatory or necrotic processes. The changes are interpreted as traumatic and mechanical. Four types of this peculiar, primary disorder of the median layer of the aorta occur, characterized by absence of concomitant tissue reactions and by a patchy or focal character: (1) necrosis of the media, as described by Wiesel, Stoerk and Epstein, in instances of acute infectious diseases; (2) idiopathic necrosis of the media, type Gsell-Erdheim, due to toxic, infectious injuries or probably avitaminic in origin; (3) disseminated necrosis of the media, type Cellina, apparently the result of senile process, and (4) traumatic elasticorrhexis of the media, type Hammer.

E. L. MILOSLAVICH.

MURDER BY DROWNING. G. BUHTZ, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18:557**, 1932.

Buhtz briefly reviews the reports of twenty-three cases of murder by drowning and describes four additional criminal drownings of young pregnant women. The analysis of all the twenty-seven cases showed a great prevalence of murdered women, the criminal act being committed by the fiancé in eleven instances, by the husband in three and by the seducing employer in one. Pregnancy was the motive in nine instances. In fourteen of the twenty-seven cases, a serious struggle preceded the violent death, as evidenced by severe bruises and signs of strangulation. In eleven instances, however, there was no evidence of any injuries; in cases similar to these, if careful and exhaustive investigations are not conducted, a suicide is summarily assumed, and a violent or criminal drowning is then readily overlooked.

E. L. MILOSLAVICH.

MICROBIOLOGIC INVESTIGATIONS IN LEGAL MEDICINE. R. E. PERL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:570, 1932.

The murdered body of a young man was found in a pool of muddy water in the close vicinity of a river, and the question arose whether the death occurred in that particular place, or whether the dead body was brought there later on. Autopsy disclosed death by drowning. Detailed bacteriologic and microscopic analysis of various samples of the water in the pool and of the lungs was made. The microscopic examinations were conclusive, since the same kind of protozoans, algae, etc., were detected in the mud as in the fluid from the lungs. The arrested suspect confessed to murder where the body was found.

E. L. MILOSLAVICH.

INTRACRANIAL ANEURYSM IN A SIX YEAR OLD GIRL. K. K. ORTMANN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:604, 1932.

The autopsy of a 6 year old girl, who died suddenly, disclosed a ruptured aneurysm, the size of a pea, in the right anterior cerebral artery, located close to the internal carotid, and accompanied by an extensive subarachnoid hemorrhage. At the age of 3 years, the child had been affected with diphtheria, and for the last six months she had complained of severe headaches. Microscopic examination showed defects of the media and inflammatory and degenerative changes. The defect in the median layer, not uncommonly observed at the point of division of arterial branches, consisted of separation of the muscular ring into single muscle bundles and fibrils, followed by complete absence of the muscular cell elements.

E. L. MILOSLAVICH.

PRESENCE OF TRACES OF METAL IN GUNSHOT WOUNDS. GERHARD BUHTZ, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:609, 1932.

Spectrographic examinations of gunshot wounds, particularly of the ring of contusion and of the smudge, in instances of wounds produced by lead bullets, invariably disclosed presence of lead. The amount of lead was proportionate to the size of the caliber and to the range of distance; the smaller the bullet and the greater the distance, the less was the quantity of the lead demonstrable. Presence of copper is characteristic of a shot discharged at close range. If the caliber of the pistol is known, one is able to determine from the amount of copper present in the inlet wound the distance from which a projectile was fired.

E. L. MILOSLAVICH.

FATAL POISONINGS WITH APIOL IN CRIMINAL ABORTIONS. HANS KRANKAUER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:626, 1932.

Reports of severe poisonings with apiol (parsley camphor) are appearing in European literature. Two fatal cases are described, involving young women who took apiol as an abortifacient. The autopsies revealed severe hemorrhagic nephritis, methemoglobinemia and fatty degeneration of renal and hepatic parenchymas. Apiol is an extract of *Fructus petroselin* and was known to the ancients as an abortifacient. It is a dangerous drug, which causes marked damages to the red blood cells and degenerative changes, particularly of fatty character, in the parenchymatous organs, especially in the liver.

E. L. MILOSLAVICH.

### Technical

THE TRYPTOPHAN TEST IN TUBERCULOUS MENINGITIS. HENRY H. LICHTENBERG, *Am. J. Dis. Child.* **43**:32, 1932.

Of seventy-eight spinal fluids subjected to the tryptophan test, all (twenty-five) from cases of tuberculous meningitis, proved either by autopsy or guinea-pig inoculation, were positive. Of the fluids showing a negative reaction, none was

demonstrated to be from patients having tuberculous meningitis. Purulent, xanthochromic and hemorrhagic fluids gave a false, but distinguishable, positive reaction. The test was of value as a diagnostic aid in the differential diagnosis of tuberculous meningitis and conditions that simulated it clinically or in spinal fluid findings.

AUTHOR'S SUMMARY.

A POTATO-EGG MEDIUM FOR THE ISOLATION OF TUBERCLE BACILLI. J. S. WOOLLEY and F. G. PETRIK, *Am. Rev. Tuberc.* **24**:596, 1931.

The authors describe a solid medium, consisting essentially of glycerinated potato extract in an egg base, for isolating tubercle bacilli from contaminated material. Similar results were obtained with either alkaline-treated or acid-treated sputum, but the alkaline method of digestion is preferred. It is necessary to seed at least twenty tubes of this medium to equal guinea-pig inoculation when the animal receives considerable amounts of material. However, positive results can be determined more readily and earlier by the culture method than by inoculation.

H. J. CORPER.

VITAL STAINING METHODS WITH NEUTRAL RED APPLIED TO NERVE DEGENERATION IN POLIOMYELITIS. W. P. COVELL and J. L. O'LEARY, *Arch. Neurol. & Psychiat.* **27**:518, 1932.

The authors point out the advantages of vital staining of sectioned nerves with neutral red. This was used either by the method of perfusion (the dye was allowed to pass into the aorta for about twenty minutes), by intravenous injection or by immersion. Often combined methods were used. The appended colored pictures show beautifully the condition of the myelin and of the axon, the vacuoles and granules of the histiocytes and the Schwann cells. The disadvantage of the method is the inability to retain permanent preparations of the experimental material, but it is a "valuable adjunct to the Marchi and Weigert procedures for the study of wallerian degeneration."

GEORGE B. HASSIN.

EGG YOLK AGAR IN CULTURE OF TUBERCLE BACILLI. R. D. HERROLD, J. *Infect. Dis.* **49**:420, 1931.

Further observations on the isolation of tubercle bacilli from sputum and other tuberculous specimens are recorded. The yolk of one egg is added to about 150 cc. of 1 per cent agar. Citric acid is added to the samples of urine and sputum to control contaminations. The egg yolk medium is particularly efficient in yielding a positive growth of tubercle bacilli from specimens found to be negative by repeated smears. The medium appears to be favorable for the rapid isolation and growth of tubercle bacilli.

EDNA DELVES.

CULTURAL AND IMMUNOLOGICAL METHODS OF DIAGNOSING INFECTIONS WITH *ENDAMOEBIA HISTOLYTICA*. B. K. SPECTOR, *J. Prev. Med.* **6**:117, 1932.

The following medium is highly satisfactory for growing *E. histolytica*: slants prepared from 3 parts of inactivated human serum that has given a negative Wassermann reaction and 1 part of 0.85 per cent sodium chloride, overlaid with a mixture of 1 part of sterile inactivated human serum that has given a negative Wassermann reaction and 6 parts of either sodium chloride or Ringer's solution. In the detection of infections with *E. histolytica*, cultural methods are probably superior to direct fecal examinations. A negative result in the complement-fixation test for *E. histolytica* does not always signify the absence of infection with *E. histolytica*, because persons vary in the formation of complement-fixing antibodies, and because treatment with emetine sometimes alters the reaction. Likewise, a positive result may not signify infection with *E. histolytica*. Skin and precipitin tests are, as yet, inadequate for the diagnosis of infections with *E. histolytica*.

AUTHOR'S SUMMARY.

# Society Transactions

## PHILADELPHIA PATHOLOGICAL SOCIETY

*Regular Meeting, March 10, 1932*

V. H. MOON, *President, in the Chair*

ECTOPIA CORDIS—DEMONSTRATION OF A CASE. CHARLES L. LINTGEN.

A moving picture demonstration was given of a new-born infant exhibiting the following congenital anomalies—ectopia cordis, talipes varus (left), bilateral cleft palate, craniorachischisis, facial hemiatrophy and an unusually short umbilical cord (4 in. [10.16 cm.]). The mother was 25 years old and had had one normal child in 1929. The child with the anomalies lived for several hours. The heart projected prominently through the anterior wall of the chest, covered only by a thin, translucent membrane. The moving pictures demonstrated its contractions and enabled one to follow the sequence of auricular and ventricular systoles.

LYMPHOSARCOMA WITH UNUSUAL METASTASES IN A THIRTEEN MONTHS OLD INFANT. P. L. DAVIS.

The lymphosarcoma took origin in the retroperitoneal chain of nodes, with metastases to the liver, to the epidural region of the cranium and, to a lesser extent, to a lung, a rib and a kidney. The cranial metastasis was the source of extremely puzzling clinical manifestations. Only at necropsy was the nature of the disease made clear. The infant was normal, born at full term and delivered normally, and was breast fed. During the first six or eight months the child did not seem as alert and active as the mother's previous children. She prided herself, however, on its apparent good nourishment, especially the round, protuberant abdomen, the contour of which was occasionally the topic for jest. When the child was 9 months old, enlargement of the cervical glands was manifest. At about the same time the mother was disturbed by frequent shrill cries of the baby during its sleep and by its fretfulness. Soon she noticed that it would fall to the right side. The right ear became more prominent than the left. In September, 1931, the child was brought to the Laryngeal Clinic of the Philadelphia General Hospital. Examination showed bulging of the posterosuperior wall of the right external auditory meatus. The tympanic membrane was not visible. Right facial paralysis of the peripheral type was noted. The laboratory findings were negative. The roentgen diagnosis was right subacute mastoiditis. The following day simple mastoidectomy was performed. No pus was obtained on opening the mastoid cells. A subsequent neurologic consultation suggested subdural abscess. Two weeks later ophthalmologic examination showed marked left exophthalmos and beginning right optic atrophy. Attention was given at this time to the cervical adenopathy. Several small doses of filtered x-rays were applied to the nodes, and under this treatment they promptly melted away.

The general condition gradually grew worse. On Jan. 18, 1932, a radical mastoidectomy was performed and necrotic bone removed from the right mastoid process. Culture showed a hemolytic streptococcus, but no pus was obtained. Neurologic signs of an involvement of the peripheral cranial nerves became obvious. Dysphagia necessitated nasal feedings. Right exophthalmos developed on Feb. 11, 1932, and dilated retinal veins and optic atrophy were prominent on examination of the eye-grounds. The child became extremely emaciated and died, Feb. 14, 1932.

After death there were found markedly enlarged retroperitoneal lymph nodes, particularly in the region of the head of the pancreas, although not invading the parenchyma of that organ. This seemed to be the primary seat of the tumor. The nodes were moderately firm and yellowish white. Their parenchyma was homogeneous and succulent, and the cut surface bulged. The chain extended cephalad as far as the hilus of the liver. The liver extended 10 cm. below the costal margin. Many irregularly rounded, firm nodules projected from its surface. On the posterolateral surface of the right lobe was a saucer-shaped concavity of large size. A cut section of the right lobe showed this concavity to be underlaid by a huge tumor mass occupying nearly the entire right lobe. Considerable central degeneration with mottled areas of hemorrhage and necrosis and a certain amount

of radial fibrous trabeculation were present. The periphery of the large mass resembled the tissue in the lymph nodes as did the cut surface of the smaller metastatic nodules. Further metastases were present as small nodules in the right kidney, the right seventh rib and the right lung. A retropleural lymph node was also involved. The epidural space of the right middle fossa was the seat of infiltration by tumor tissue to a thickness of 2 cm. The inner plate of the skull over the middle ear and mastoid regions was eroded, and the operative wound was in direct communication with the tumor mass. The right orbital fossa was also invaded. Through this epidural tumor mass the majority of the right cranial nerves must necessarily have passed to their exit from the skull.

Microscopically, the tumor cell throughout was the lymphocyte of adult form. In other words, the growth was a lymphosarcoma; subgroup, malignant lymphocytoma. Sections through the retroperitoneal nodes showed the architecture to be completely obliterated, the nodes being transformed into homogeneous cell masses. The stroma consisted of a delicate reticulum that divided the tumor cells into faintly discernible alveoli. The capsule of the node was infiltrated by tumor cells. The liver showed massive replacement of parenchyma by lymphocytic invasion. The smaller nodules were densely cellular; their immediate periphery showed tumor clumps within lymphatic channels and portal vein radicals, thus establishing a dual metastatic spread. The massive lesion in the right lobe of the liver was the seat of considerable central degeneration and fibrosis. The extent of the fibrosis suggested that the metastatic lesion here was of comparatively long duration, undoubtedly months. The spleen was uninvolved in the process. Sections through the skull overlying the right middle ear and mastoid process showed extreme infiltration by tumor cells, which were undoubtedly blood-borne to this situation, as evidenced by cell masses in veins and blood sinusoids of the marrow cavity. A rather marked fibrous osteitis was present, a reaction to the presence of tumor.

ALYMPHOID LEUKOPENIA AND PURPURA HEMORRHAGICA WITH AN ACUTE MILIARY EXACERBATION OF CHRONIC PULMONARY TUBERCULOSIS.  
HAROLD W. JONES, LEANDRO M. TOCANTINS and BAXTER L. CRAWFORD.

A girl, aged 15, was admitted to Jefferson Hospital in critical condition from epistaxis, long, profuse menstrual periods and a transfusion reaction. There was no history of tuberculosis in the family or in the girl. There was, however, a history of bruising and nosebleed in both parents and of nosebleed in the girl before her menstrual periods started. Three months before admission bruising was extensive. In spite of repeated blood transfusions, liver, liver extract, iron, a diet high in vitamin and high in protein, and roentgen treatment of the long bones and spleen, the condition became progressively worse; evidences of left-sided pneumonia developed, and the patient died two and a half months after admission.

From the day of admission there was marked leukopenia. The white blood cell count varied from 1,150 to 400, the smaller count being present at the termination of the illness. Contrary to the usual finding, the lymphocytes were greatly diminished, being always below 10 per cent and sometimes absent, and the granulocytes were more than 90 per cent and at times 100 per cent of the white blood cells.

*Autopsy.*—Slight generalized jaundice and marked anemia were found, with subcutaneous and subserous petechial hemorrhages and hemorrhages in the ovaries. There was a rather extensive tuberculous lesion in the left lung. In the apex were a small, contracted scar and a small encapsulated, caseous nodule, with numerous miliary and conglomerate foci throughout the lung; no cavitation was seen. The right lung also contained many miliary tubercles. The spleen weighed 80 Gm.; on microscopic examination the fibrous tissue was slightly increased, and the follicles were atrophic. No normal follicles with definite germinal centers were observed. Several tuberculous ulcers were found in the ileum. The liver weighed 1,230 Gm., and microscopic examination revealed considerable degeneration of the epithelial cells and beginning fibrosis with proliferation of the bile ducts, but very little evidence of leukocytic infiltration. The bone marrow of both the tibia and the femur was found to be entirely fatty, and microscopic examination



revealed that it was hypoplastic, with no evidence of hyperplasia of the hematopoietic cells. No megakaryocytes were found.

(The history of the association of tuberculosis and purpura from an experimental and clinical standpoint is discussed. It is set forth that tuberculosis is commonly found in cases of purpura hemorrhagica, and that many of the fulminating cases, so-called essential types, would be found to be associated with tuberculosis if a postmortem examination were made.)

The interesting points are a family history of bruising, nosebleed and long menstrual periods, the history of nosebleed in the patient before menstruation began, of long profuse bleeding when it became established, and of bruising and nosebleed before there was clinical evidence of tuberculosis. There was a comparatively high platelet count in the absence of megakaryocytes in the bone marrow. The bleeding time was greatly prolonged (thirty-one minutes), with a platelet count of about 100,000. A marked leukopenia was present with a high granulocyte count and an absence or a great reduction of lymphocytes.

We are unable to explain the phenomena presented on the basis of our clinical and pathologic findings. We suggest the name "alymphoid leukopenia" for this condition. Such a title is as applicable in this case as is "agranulocytic angina" or "anemia" for the condition described by Schultz and others.

#### *Regular Meeting, April 14, 1932*

The Annual Conversational Lecture was given by Dr. Arthur I. Kendall, of Northwestern Medical School: "Observations on the Significance of Filtrable Bacteria."

#### *Regular Meeting, May 19, 1932*

#### COMPARATIVE PATHOLOGY OF ARTERIOSCLEROSIS. HERBERT FOX.

(A lantern slide demonstration of arterial lesions occurring in many species of mammals, birds and reptiles was given. The material was largely drawn from necropsies at the Philadelphia Zoological Garden.)

#### GASTRIC ULCER IN CAPTIVE WILD ANIMALS. HERBERT L. RATCLIFFE.

Ulcerative lesions of the mucosa and wall of the stomach and duodenum, closely similar in gross and microscopic appearance to those in man, are found in many types of mammals. The records of the Laboratory of Comparative Pathology of the Philadelphia Zoological Society show that this disease has occurred in approximately 1 per cent of 5,000 mammals. Carnivora and Primates are most frequently affected, but gastric ulcer has been seen also in Marsupialia and Artiodactyla. Birds have not been affected. The ulcers have much the same anatomic distribution, and the behavior is also much the same as in man. Fatal hemorrhage, perforation with peritonitis or healing with radiating scar formation has been observed. The specimens exhibited illustrated these features.

#### RUPTURE OF THE ESOPHAGUS. A REPORT OF TWO CASES. A. D. WALTZ, CLARA I. DAVIS and R. P. REGESTER.

A thorough search of the literature on rupture of the apparently healthy esophagus reveals reports of only thirty-four cases. The earliest is that of Admiral Baron von Wassenair reported by Boerhaave in 1724. Full reviews of the subject may be obtained from articles by E. J. McWeeney (*Lancet* 2:161, 1900) and H. T. Williams and William Boyd (*Surg., Gynec. & Obst.* 42:56, 1926).

CASE 1.—A colored woman, aged 55, was admitted to Delaware County Hospital on Jan. 16, 1931, in the service of Dr. R. P. Regester. The patient had seldom been sick, having had only one (slight) attack of indigestion one year before. She said she did not use alcohol and tobacco. Her illness, lasting thirteen days, began one evening with acute pain in the upper part of the abdomen, following a meal of stewed beef. The patient took magnesium sulphate and two acetylsalicylic

acid tablets, which were immediately vomited. Vomiting continued intermittently all night and the following morning, but the pain disappeared. The vomitus was greenish. There was no hematemesis. Examination revealed an obese colored woman in semistupor. The pupils reacted normally to light. The breath had a foul odor, and the pharynx was slightly congested. The neck was normal. There were no adventitious signs in the lungs. The heart was regular, rapid and difficult to outline. The abdomen was tender and slightly rigid in the hypochondrium. The blood pressure was 200 systolic and 110 diastolic. A tentative diagnosis of acute disease of the gallbladder was soon changed to meningococcic meningitis. The patient grew worse and died suddenly on Jan. 29, 1931. At autopsy, in addition to the usual lesions of meningococcic meningitis, the esophagus was found ruptured (4 by 1.5 cm.) on its left side above the diaphragm. The surrounding tissue showed no inflammatory change, but the edges of the ruptured portion were necrotic. The left pleural cavity contained 500 cc. of thin, dark red fluid similar to that found in the stomach.

CASE 2.—A white woman, aged 27, was admitted to Delaware County Hospital, Feb. 7, 1932. The patient had been delivered one month previously of a child who died of syphilis and a hemorrhagic disorder. The onset of the present illness occurred three days after a second injection of 0.3 Gm. of neoarsphenamine. The patient felt as though she were becoming "wild" and returned home screaming. At this time the family physician carried on artificial respiration. The left pupil was dilated. She felt well the following morning, but fell asleep that afternoon on taking  $\frac{1}{4}$  grain (0.016 Gm.) of phenobarbital and 1 grain (0.06 Gm.) of phenobarbital sodium. She presented the picture of deep drug narcosis. The entire body was covered by a macular rash. The heart was fibrillating. The heart sounds were harsh, and a few râles could be heard in both bases. The abdominal findings were negative except for slight distention of the abdomen. All reflexes were absent. The patient never regained consciousness, and died nine and one-half hours after admission to the hospital. Autopsy soon after death showed a linear rupture of the esophagus 7 cm. in length just above the diaphragm. There was 350 cc. of dark brown fluid in the left pleural cavity, also similar fluid in the stomach. The right pleural cavity was normal. The remaining organs show marked toxic degeneration.

#### A SEVERE CASE OF ALKALOSIS. A. I. RUBENSTONE and JACOB LEVY.

A Russian Jewess, 29 years old, was delivered of a  $1\frac{1}{2}$  pound (453.6 Gm.) fetus at the end of six and one-half months of gestation. She had shown symptoms of toxemia throughout this and a previous pregnancy. Five days after delivery she had lapsed into stupor from which she aroused at intervals. Examination of the blood revealed a carbon dioxide content of from 108 to 114 per cent by volume. The blood chlorides amounted to 384 mg. and the nonprotein nitrogen to 63 mg. per hundred cubic centimeters. The stupor deepened in spite of energetic treatment. Marked cyanosis and muscular twitching developed shortly before death. Attention is called to the fact that persons exhibiting symptoms often referred to acidosis may have alkalosis.

#### A CASE OF PRIMARY SQUAMOUS CELL CARCINOMA OF THE THYROID GLAND. A. I. RUBENSTONE and D. R. MERONZE.

A white woman of 55 years complained, on Nov. 8, 1930, that for several months she had been conscious of a "tightening" sensation on swallowing. This had increased until she found it difficult to swallow liquids. Physical examination revealed an enlarged, irregular left lobe of the thyroid gland, which was hard and nodular, with deviation of the trachea to the right and compression, and paralysis of the left vocal cord. On December 10, approximately three months after the onset of the symptoms, she was admitted to Mount Sinai Hospital. At this time loss of weight was noted. The trachea was fixed, and esophagoscopy revealed an infiltrative lesion of the upper part of the esophagus, but without ulceration. Biopsy proved the lesion to be a squamous cell carcinoma. The patient's condition became rapidly worse. She died on Jan. 24, 1932, one week after an emergency tracheotomy.

Primary carcinoma of the thyroid gland is a relatively uncommon condition. In a series of 6,535 thyroid glands operated on and examined at Lahey Clinic in Boston between 1916 and 1930, 187, or 2.86 per cent, showed primary malignant growth. In this group only one showed a squamous cell carcinoma. The tumor presented here, we believe, is a primary squamous cell carcinoma.

At autopsy, the area normally occupied by the left lobe of the thyroid gland was filled with a large quantity of necrotic, grayish material, alternately composed of soft and hard masses of tissue, in the midst of which were several hard, white, homogeneous areas. The left sternocleidomastoid muscle was adherent to this area. The muscle was hard and apparently infiltrated, forming a firm mass with the regional blood vessels and lymph nodes. The right side grossly appeared relatively normal, but on section it was also seen to be partly replaced by a hard, white, homogeneous tissue of the same kind as that seen on the left side. A large mass of glands on the right side of the trachea were hard in consistency and on section showed a white, homogeneous appearance. The tumor mass infiltrated and projected into the upper part of the esophagus from without. The lower portion of the esophagus was normal. An obstruction was met, on entering the esophagus from above, at the level of the cricoid cartilage. This obstruction was due to a deviation of the pharynx to the left, causing narrowing and kinking of the esophagus. The first two tracheal rings felt softer than normal, and there was marked narrowing of the lumen in this region, caused by deviation of the trachea to the right. The rima glottidis was closed by a swelling in the region of the left arytenoid cartilage. On incision, the entire mechanism of the larynx was distorted by a mass involving the left side. The mass bulged the wall forward, filling the lumen and displacing the left vocal cord upward and posteriorly. This mass was in the thyroid cartilage and was part of the mass described as replacing the left lobe of the thyroid gland. It had a hard, white, homogeneous appearance on section. It had destroyed a large part of the cartilaginous tissue. There was no break in continuity in the mucosa lining the larynx. Metastatic lesions were also found infiltrating the spleen.

Histologically, the tumor involved the thyroid gland, larynx, mediastinal contents and esophagus. The cells were polyhedral and flattened in shape, such as are seen in squamous epithelial tumors. Their nuclei were large, contained nucleoli and often showed mitoses. Occasionally they formed small, glandlike structures, but in the great majority of instances they formed solid cores or lobules. Prickles could not be identified, but there were large numbers of epithelial pearls. In some regions these cells penetrated connective tissue, itself infiltrated with round cells and polymorphonuclear leukocytes. One section from the thyroid gland showed the malignant growth infiltrating normal thyroid tissue.

From the clinical report it will be seen that the tumor did not spring from the esophagus or from the trachea. This opinion is further substantiated by the gross findings at autopsy in which no lesion was seen arising in the esophagus or in the remainder of the gastro-intestinal tract or in the pulmonary system. The tumor masses compressed the esophagus and trachea from without. No other tissue where a malignant condition was found in this particular case could be considered a primary site. The integrity of the skin, which was carefully examined, precludes an assumption that it originated from the integument of the neck. There still remains the possibility of an origin from a branchial remnant.

The diagnosis of branchiogenic carcinoma after it has advanced to the cervical lymph nodes may be difficult. It should be emphasized that the usual form of the tumor is a cystic growth in and about which are carcinomatous areas, and that most cases are encountered when the cysts are recognizable, and when a squamous cell cover is present or predominant. We believe that this is not a tumor of this sort because it lacks the cystic quality of the usual branchiogenic tumor, because none but epithelial tissues were found therein, and because from the earliest symptoms the major tumor mass was in the thyroid gland. Having eliminated other local sites as possible sites of primary squamous cell carcinoma, we conclude that the case is an instance of primary squamous cell carcinoma of the thyroid gland, probably arising from the thyroglossal duct.

## Book Reviews

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**Recent Advances in Pathology.** By Geoffrey Hadfield, M.D., F.R.C.P. (Lond.), Professor of Pathology in the University of London, Pathologist to the Royal Free Hospital, and Lawrence P. Garrod, M.A., M.B., B.Ch. (Camb.), M.R.C.P. (Lond.), Bacteriologist and Lecturer in Bacteriology, late Demonstrator of Pathology, St. Bartholomew's Hospital. Price, \$3.50. Pp. 392, with 67 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

The series on "Recent Advances in Pathology" has grown rapidly. It now includes twenty-five volumes, representing different phases of medicine. All the volumes are products of British authors. The volume by Hadfield and Garrod presents a survey of recently acquired knowledge bearing on fundamental problems connected with a number of specific diseases. The first chapter deals with the reticulo-endothelial system, its composition, functions and relation to immunity, metabolism of bile pigment and certain splenomegalies. Then comes a review of tissue culture and its uses. The next four chapters are concerned with research on cancer: carcinogenesis by irritants, transplantation, filtrable tumors, tumor metabolism, heredity and the actions of roentgen rays and radium. A clear account is given of the discovery and properties of the vitamins, with a discussion of the deficiency diseases, hypervitaminosis and calcium metabolism. In the realm of the cardiovascular system, endocarditis, especially the rheumatic and infective types, and diseases of the arteries receive special attention. The account of experimental endocarditis does not stress sufficiently the establishment by Rosenow and other experimenters of endocarditis by intravenous injections of bacterial suspensions only. The respiratory system is represented by surveys of pneumonia, principally its experimental production, of primary carcinoma of the lung, of the pneumoconioses and of anoxemia. The chapter on the digestive system deals with peptic ulcer, necrosis of the liver, the changes in the pancreas in diabetes and the islet tumors. The recent work on Bright's disease receives consideration. In the case of the central nervous system, the newer work on the neuroglia and the glioma group of tumors as well as on the problem of encephalitis is reviewed. The last chapter is devoted to the thyroid, the parathyroids and the suprarenals. There are an index of subjects and one of authors. At the end of each section is a list of references for guidance to important recent publications. In the text are helpful summaries and tabulations. The authors have succeeded in writing good reviews of recent significant work in the fields mentioned.

**Entstehung, Erkennung und Behandlung innerer Krankheiten.** By Dr. Ludwig Krehl, Professor in Heidelberg. Erster Band. Die Entstehung innerer Krankheiten: Pathologische Physiologie. Fourteenth edition. Price, 39.60 marks. Pp. 716. Berlin: F. C. W. Vogel, 1932.

This is Krehl's "Pathologische Physiologie" dressed up in a new garb as "Die Entstehung der inneren Krankheiten." The preceding edition first bore this new title and was published as one of three volumes designed to embody the principles of the practice of internal medicine. Two are available; the third, devoted to therapy, will soon be at hand. This volume has had a long and successful career. It was first published in 1893, as "Grundriss der allgemeinen klinischen Pathologie." Translations soon appeared in French, Russian, Polish and other languages; the third German edition was translated into English by Hewlett; a subsequent translation into English was made by Beifeld from the seventh German edition. It is singular that the author's prefaces contain no statements reflecting satisfaction with his accomplishment. The preface for this last edition is a reprinting of that in the thirteenth, to which a short note has been added. In the latter, he says,

no one knows as he does how far the book falls short of his ideals. This is certainly an unusual humility, judged by his results during almost forty years of steadily improving an achievement successful in the first place. In an introductory note to Hewlett's translation (1906), Osler commented on the position the "Clinical Pathology" then occupied: "Not that it needs any words of commendation," an encomium even more justifiable now.

In the first edition the chapters were on the circulation, blood, respiration, digestion, metabolism, fever, urinary excretion and the nervous system. These have been retained throughout the numerous editions and have gradually been added to, so that now there are also chapters on endogenous disease and constitution, infection and immunity, origin of infectious diseases, energy exchange, water and salts in the body (mainly a discussion of edema) and coordinate functioning of the organs. Changes from the edition of two years ago are not significant, simply those one expects in a well established book maintaining its position as a staple commodity in medical literature: A paragraph has been added here and there which discusses the results of recent investigation; there are occasional new sentences to express the author's previous opinion in more vigorous terms; about thirty pages have been added, there is a more comprehensive list in the table of contents of matters considered in each chapter, and all of the familiar aspects of arrangement and printing have been retained.

Overcrowding of undergraduate instruction with objective description, summaries of facts and laboratory methods has left little room in the student's regimen for textbooks largely devoted to principles. This probably accounts for some restriction, indeed deplorable, of the rôle Krehl's book has upheld, so pre-eminently a reference work for clinicians and an exegesis for the physician's hour of leisure. In the domain of the purely morphologic, great difficulty has always been experienced in so presenting disease that its somatic structural modifications may be visualized. To comprehend the manner in which disease in its functional disturbances also involves the entire body is an even larger undertaking. The chief value of this, Krehl's masterpiece, is its dedication to that object in so large a measure. For this reason alone its use by medical students should be extended. In other fields its value has been fully realized.

**The Sign of Babinski: A Study of the Evolution of Cortical Dominance in Primates.** By John F. Fulton, Sterling Professor of Physiology in the Yale University School of Medicine, and Allen D. Keller, Professor of Physiology and Pharmacology in the School of Medicine, University of Alabama. Price, \$5. Pp. 165, with 66 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

This research presents an experimental approach to a major pathologic problem which is unusual in method and results. The method employed is unusual because of its cost in time and in money; the results achieved are unusual because of the rare competence of the authors in neurophysiology, in surgical technic and in critical acumen. The Babinski reaction is one of the most important signs employed in clinical neurology; just what it means is still controversial, and this study takes one far toward a solution of the problem.

The account begins with a reproduction and translation of Babinski's original description of 1898. The scanty comparative data in the subsequent literature indicate that the true Babinski reflex is seen only in the primate foot. The authors have studied it experimentally in several species of long-tailed monkeys, twenty-two baboons, two gibbons and nine chimpanzees, with a critical survey of previous experimental work on primates. These experiments included semi-section and transection of the spinal cord at various levels, electrical stimulation of the cortex, ablation of one or both leg areas of the cortex, complete removal of one cerebral hemisphere and various combinations of these operations. In the spinal monkey the Babinski phenomenon, when present, is part of a generalized flexion reflex. In these monkeys no Babinski reflex is obtained from cortical destruction of the pyramidal tract; the reaction is thus not solely an affair of the

pyramidal pathways. In the baboon and gibbon, there is a progressive change in the conditions which evokes this sign until in the chimpanzee a true Babinski response can be obtained after ablation of the leg area of the cortex. Other signs of degeneration of the pyramidal tract are described and discussed. "In the higher primates the sign of Babinski is associated with isolated destruction of the pyramidal pathways." In the large apes the cortical leg area exerts some measure of control over the musculature of the same side of the body.

The clinical implications are discussed, especially in traumatic cerebral diplegia, Little's disease and spinal shock. "It is obviously significant that a cortical lesion in the higher primates resembles spinal 'shock' more closely than in the lower primates, and it serves to illustrate in dramatic fashion the process of encephalization in primate evolution. In man the dominance of the hemisphere is so complete that simultaneous destruction of both foot areas is virtually equivalent, as far as motor power of the lower extremities is concerned, to spinal transection." The book closes with an appendix on surgical technic, a bibliography (114 titles) and an index.

**Techniques de laboratoire appliquées aux maladies de l'appareil digestif et de la nutrition.** By Marcel Labbé, Membre de l'Académie de Médecine, Professeur de Clinique médicale à la Faculté de Médecine de Paris, Henri Labbé, Professeur agrégé, Chef de travaux pratiques de Chimie à la Faculté de Médecine de Paris, and Floride Nepveux, Chef de Laboratoire à la Faculté de Médecine de Paris. Paper. Price, 140 francs. Pp. 886, with 141 illustrations and charts and 6 colored tables. Paris: Masson & Cie, 1932.

This book is the outcome of a course given by the authors since 1900 at the medical faculty of the University of Paris. One of the authors is a clinician; the others are laboratorians. The responsibility for the various chapters is divided. The book gives a great deal more than is indicated by the title. It represents, in reality, a complete treatise on physiologic chemistry in 360 pages, as well as a detailed outline of laboratory technic in the remaining 507 pages.

The theoretical chapters, which present the important modern conceptions on the various phases of the subject, are made particularly interesting by the critical attitude of the authors. The pros and cons of each controversial question are followed by the personal views of the writers. In the chapters devoted to technical procedures, the critical attitude is still more pronounced. It is quite apparent that their preference for certain procedures and the rejection of others are based on personal experience of many years. In other words, the book is not merely a compilation of various laboratory tests, but a critical evaluation of procedures which stood the acid test of clinical application.

The examination of the gastric and duodenal contents is discussed in 17 and 18 pages, respectively, followed by 264 pages devoted to an extremely thorough presentation of tests employed in the examination of urine. The authors lay great emphasis on the value of quantitative chemical analysis of urine for the study of metabolic changes. When the reviewer compares the space given in this book to urinalysis with the tendencies in the majority of modern textbooks on laboratory procedures to emphasize the value of the chemical examination of the blood at the expense of the urinalysis, he welcomes the attitude of the French authors and believes with them that much direct information can be gained by the study of urine which is nowadays frequently arrived at by using roundabout, expensive ways.

In the chapter of 60 pages devoted to feces, as well as in the remainder of the book, the bacteriologic phases are considered merely in passing. In the last chapter the chemical analysis of blood is discussed in 134 pages. For some reason, which is not apparent to the reviewer, creatine and creatinine have been omitted from this technical chapter, though they were discussed in 3 pages in the theoretical part.

The size of the book is unnecessarily increased by numerous repetitions in the technical chapters of theoretical discussions of questions already exhaustively presented in the theoretical part of the book. An almost literal repetition of a paragraph on page 572 and a few errors in the numbering of figures were noticed. The absence of a subject index detracts considerably from the value of the book.

**La maladie de Boeck: Sarcoides cutanées bénignes multiples.** By Dr. A. Kissmeyer, Médecin de l'Institut Finsen. Preface by Dr. J. Darier. Pp. 147, with 67 illustrations. Copenhagen: Levin & Munksgaard, 1932.

Since Boeck, in 1897 at Christiania, first described the skin disease which bears his name, it has been a matter of great speculative etiologic interest to dermatologists.

Kissmeyer, who has devoted several years to the study of cutaneous and other sarcoids, bases his monograph on 28 personal cases of Boeck's disease and 250 other cases reported in the literature. Under the caption of benign cutaneous sarcoid, he includes Besnier's lupus pernio, Pautrier's angiolupoid and a sarcoidic erythrodermia which seems to correspond to Darier's "erythematous tuberculids." Other forms, such as granuloma annulare, are questionably omitted. Generally, he classifies Boeck's disease as a type of chronic infectious granuloma which involves not only the skin but also the mucosae, lymphatic system, parenchymatous organs, skeleton and the like. The usual clinical types—large and small nodular, disseminated and diffuse—are described.

The 23 short chapters which make up the monograph include the clinical descriptions of the various types and discussions of the various opinions held by different investigators.

In the chapter on histology, the principal point made is that the absence or only slight degree of lymphocytic reaction around the disease foci is almost pathognomonic of sarcoid, as well as the distinct limitation toward the surrounding tissues.

The chapters of greatest interest are those dealing with the etiology. Kissmeyer reviews Boeck's changing attitude toward what he considered the nature of the disease, and also the views of Darier, Kyrle and others who supported or denied its tuberculous origin. Some of the patients studied by these authors were unquestionably tuberculous apart from the sarcoid. In only 6 of 150 cases critically studied by Kissmeyer was there an unquestionable tuberculosis. Therefore, on this account, as well as because of his failure in any of his own cases to demonstrate the tubercle bacillus by any of the known clinical or biologic tests, he expresses the opinion that when a cutaneous disease the virus of which is not yet known gives a negative or weak reaction with great regularity, a tuberculous origin is improbable. In his introduction, however, Darier points out that this does not exclude a filtrable form of the bacillus.

The fact that the author's tests of the blood and diseased tissues for the tubercle bacillus were 100 per cent negative is strong evidence against the view, now commonly held in textbooks and elsewhere, that Boeck's sarcoid of the skin is of tuberculous origin. He considers his view strengthened by the fact that in his cases arsenic was the drug which gave the best results; it is of little value in cutaneous tuberculosis.

Kissmeyer further finds that the cutaneous and osseous manifestations approximate the sarcoids to lepromas; there are as many clinical facts common to sarcoid and leprosy as to sarcoid and tuberculosis.

As a conclusion, Kissmeyer thinks that in the absence of a specific virus Boeck's disease cannot yet be definitely classified among the chronic infectious granulomas. It should be placed between tuberculosis and leprosy.

Kissmeyer's monograph, while it leaves matters still unsettled, nevertheless is an important contribution which must be referred to by all future workers on this puzzling condition.

**Special Cytology. The Form and Functions of the Cell in Health and Disease. A Textbook for Students of Biology and Medicine.** By Various Contributors. Edited by Edmund V. Cowdry, Washington University, St. Louis. In 3 volumes. Second edition. Fabrikoid. Price, \$30 per set. Pp. 1838, with 757 illustrations. New York: Paul B. Hoeber, Inc., 1932.

The first edition of this work appeared in the early part of 1928 and was reviewed favorably in the *ARCHIVES* (5:1152 [June] 1928). The second edition is an impressive improvement and expansion of the first. The number of volumes has been increased from two to three, and the price accordingly from \$20 to \$30 for the set. The original sections have been revised by their authors, and in some cases have been completely rewritten in order to reflect more adequately the recent advances of knowledge in the special fields. The section on the lymphocytes and plasma cells by the late Alexander A. Maximow has been revised by W. Bloom. The major part of the increase in the size of the second edition is due to the inclusion of new sections to cover subjects not previously considered, namely: human uterine gland cells by C. W. Bartelmez and R. R. Bensley; the gastric glands by R. R. Bensley; cartilage and bone by A. W. Ham; the spleen by P. Kyes; ciliated epithelium by A. M. Lucas; the cornea and lens by Ida Mann; architecture of nerve cells as revealed by microdissection by G. S. de Renyi; the suprarenal bodies by J. M. Rogoff; bone marrow by Florence R. Sabin; the teeth by J. Schour; the hair by Mildred Trotter, and the capillaries by A. Krogh and B. Vimtrup. The work now contains forty-four sections by forty-six contributors, about twenty-six of whom are anatomists. Each section is provided with a list of contents at the beginning and a bibliography, sometimes quite extensive, at the end. Through the three volumes the pagination runs consecutively. The third volume contains the index. The main topics in each volume are given on its back, and it would have been helpful to the reader if the pages included in the volume had been given also. The illustrations, almost exclusively black and white, are adequate and commendable. Elaborate descriptions of specialized technical details are avoided, but the results of the intensive study of the cells and cellular activities by modern technics are presented in authoritative fashion by writers with intimate, first hand knowledge each of his own subject. Embryogenic cytology is not considered. Attention is directed mainly to the principal types of cells in the postembryonal human body. The work should be available to all students of normal and abnormal cytology.



## Books Received

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FUNGUS DISEASES: A CLINICO-MYCOLOGICAL TEXT. By Harry P. Jacobson, M.D., Attending Dermatologist and Member of the Malignancy Board, Los Angeles County General Hospital. With Introduction by J. Frank Schamberg, M.D., Professor of Dermatology and Syphilology, Graduate School of Medicine, University of Pennsylvania, and Howard Morrow, M.D., Clinical Professor of Dermatology, University of California Medical School. Price, \$5.50. Pp. 290, with 153 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

VITAMINS: A SURVEY OF PRESENT KNOWLEDGE. Compiled by a Committee appointed jointly by the Lister Institute and Medical Research Council. Medical Research Council, Special Report Series, No. 167. Price, 6 shillings 6 pence, net. Pp. 319. London: His Majesty's Stationery Office, 1932. (It can be obtained from The British Library of Information, 5 East Forty-Fifth Street, New York.)

THE THIRD PANDEMIC OF PLAGUE IN EGYPT: HISTORICAL, STATISTICAL AND EPIDEMIOLOGICAL REMARKS ON THE FIRST THIRTY-TWO YEARS OF ITS PREVALENCE. By A. W. Wakil, M.B., Ch.B. (Cairo), Dip. Hyg. (Camb.), D.T.M. & H. (Camb.), Assistant Professor of Hygiene and Preventive Medicine Faculty of Medicine, Cairo. Pp. 169. Cairo: The Egyptian University, 1932.

CLASSIC DESCRIPTIONS OF DISEASE. By Ralph H. Major, M.D., Professor of Medicine, University of Kansas School of Medicine. Price, \$4.50. Pp. 630. Springfield, Ill.: Charles C. Thomas, 1932.

THE SPUTUM: ITS EXAMINATION AND CLINICAL SIGNIFICANCE. By Randall Clifford, M.D., Associate in Medicine, Peter Bent Brigham Hospital; Assistant in Medicine, Harvard Medical School. Price, \$4. Pp. 167, with 21 figures and 7 plates in color. New York: The Macmillan Company, 1932.

REPORT ON CHRONIC DISEASE IN NEW JERSEY. Made in Accordance with Joint Resolution No. 3, P.L. 1931. State of New Jersey, Department of Institutions and Agencies, Trenton, 1932.

UNITED FRUIT COMPANY MEDICAL DEPARTMENT: TWENTIETH ANNUAL REPORT. Boston, 1931.

CLINICAL ENDOCRINOLOGY OF THE FEMALE: By Charles Mazer, M.D., F.A.C.S., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, University of Pennsylvania; Gynecologist to Mount Sinai and Northern Liberties Hospitals, Philadelphia; and Leopold Goldstein, M.D., Demonstrator of Obstetrics, Jefferson Medical College; Assistant Gynecologist to Mount Sinai Hospital. Price, \$6, net. Pp. 518, with 117 illustrations. Philadelphia: W. B. Saunders Company, 1932.

AMERICAN ILLUSTRATED MEDICAL DICTIONARY: A COMPLETE DICTIONARY OF THE TERMS USED IN MEDICINE, SURGERY, DENTISTRY, PHARMACY, CHEMISTRY, NURSING, VETERINARY SCIENCE, BIOLOGY, MEDICAL BIOGRAPHY, ETC. By W. A. Newman Dorland, M.D., Member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association. Edition 16. Flexible and stiff binding, plain, \$7, net; thumb index, \$7.50, net. Pp. 1,493 with 941 illustrations and 279 portraits. Philadelphia: W. B. Saunders Company, 1932.

## LIPOIDGRANULOMATOSIS (TYPE, HAND-SCHÜLLER-CHRISTIAN)

REPORT OF A CASE

WILLIAM CHESTER, M.D.

AND

V. H. KUGEL, M.D.

NEW YORK

Rowland<sup>1</sup> in 1928 collected twelve instances of a disease described under various titles in the literature and characterized by the symptom complex of defects in the membranous bones, exophthalmos and diabetes insipidus. He entitled the disease "Schüller-Christian's syndrome." To these he added two striking cases of his own, one of which was studied post mortem. Hand<sup>2</sup> in 1893, under the title of "Polyuria and Tuberculosis," described this symptom complex in a boy of 3 years who, in addition to exophthalmos and polyuria, showed at necropsy yellow nodules in the defects in the internal and external tables of the skull, grayish nodules in an enlarged liver and enlargement of the spleen. Only the nodules in the liver were studied microscopically; they revealed chronic inflammatory granulation tissue. In the absence of significant criteria, the condition was named in the pathologic report tuberculosis, although syphilis was also considered. The author noted, however, that he believed the lesion was neither that of tuberculosis nor that of syphilis. Kay in 1905 reported an instance of "acquired hydrocephalus with atrophic bone changes, exophthalmos and polyuria" in a boy of 7 years, which he ascribed to a tumor at the base of the brain. Schüller<sup>3</sup> in 1915 described two cases, in an article entitled "A Peculiar Syndrome of Dyspituitarism." The first was that of a girl of 3 years with the same symptom complex. He thought the condition was due to a disturbance of the pituitary gland. In the second instance, the patient suffered, in addition, from dystrophia adiposogenitalis. Schüller attributed the condition to a tumor at the base of the brain.

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From the Medical Service of Montefiore Hospital.

1. Rowland, R. S.: *Arch. Int. Med.* **42**:611, 1928.

2. Hand, A.: *Arch. Pediat.* **10**:673, 1893; *Am. J. M. Sc.* **162**:509, 1921; *Proc. Philadelphia Path. Soc.* **16**:282, 1891-1893.

3. Schüller, A.: *Wien. med. Wchnschr.* **71**:510, 1921; *Fortschr. a. d. Geb. d. Röntgenstrahlen* **23**:12, 1915-1916; *Brit. J. Radiol.* **31**:156, 1926.

Christian <sup>4</sup> in 1919, under the title, "Defects in Membranous Bones, Exophthalmos, and Diabetes Insipidus—An Unusual Syndrome of Dyspituitarism," reported the case of a girl of 5 years. None of the cases except that of Hand came to autopsy. The other authors ascribed this unusual symptom complex to dyspituitarism or to a tumor at the base of the brain. In view of the apparent historical priority it has been suggested <sup>5</sup> that this symptom complex be designated as Hand's disease.

Subsequently, instances of this disease were reported by numerous investigators. The literature to 1930 has been reviewed by one of us in a previous article,<sup>5</sup> and to 1931, by Moreau.<sup>6</sup> Further instances have been described by Chiari,<sup>7</sup> Sosman,<sup>8</sup> Frumann-Dahl and Forsberg,<sup>9</sup> and Ighenti.<sup>10</sup>

Sosman mentioned that several instances have been reported<sup>11</sup> in which the symptom complex of Hand's disease was present and, in addition, an involvement of the cranial bones which was similar roentgenologically to osteitis fibrosa. In a previous communication <sup>5</sup> were reported changes in the vertebrae, found roentgenologically, which on microscopic examination proved to be similar to those found in osteitis fibrosa (*zusammengesetzte Bälkchen* of Freund).<sup>12</sup>

The observations in the cases studied anatomically are listed in table 1. In an earlier publication,<sup>5</sup> two cases of disturbed lipid metabolism that came to autopsy were described, and it was concluded that in these cases, as well as in all the allied cases of Hand's disease, the basic lesion was a chronic, noninfectious, abacterial, inflammatory granuloma due to the deposition of various lipid substances in the involved tissues. This lesion was termed "lipoidgranuloma," and it is believed

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4. Christian, H. A.: Defects in Membranous Bones, Exophthalmos and Diabetes Insipidus, Contributions to Medical and Biological Research, New York, Paul B. Hoeber, Inc., 1919, vol. 1, p. 390.

5. Chester, W.: Virchows Arch. f. Path. Anat. **279**:561, 1930.

6. Moreau, J.: Arch. franco-belges de chir. (supp.) **32**:697, 1931.

7. Chiari, H.: Ergbn. d. allg. Path. u. path. Anat. **24**:396, 1931.

8. Sosman, M. C.: Am. J. Roentgenol. **23**:581, 1930; J. A. M. A. **98**:110, 1932.

9. Frumann-Dahl, J., and Forsberg, R.: Norsk mag. f. lægevidensk. **92**:523, 1931; abstr., J. A. M. A. **97**:820, 1931.

10. Ighenti, W. K.: Virchows Arch. f. path. Anat. **282**:585, 1931.

11. Schoen, R.: München. med. Wchnschr. **71**:1713, 1924. Heard, J. D.; Schumacher, F., and Gordon, W. B.: Am. J. M. Sc. **171**:38, 1926. Sophian, A.: J. A. M. A. **95**:483, 1930. Slauch, A., and Donalies, H.: Med. Klin. **26**:459, 1930.

12. We are at a loss, as is the author himself, to interpret Wassiljeff's case. It can hardly be termed a case of Hand's disease, though the osteosclerotic process in the bones may well be subsequent to lipid deposits.

TABLE 1—Recorded Cases of Lipoidgranulomatosis (Hand-Schuller-Christian Type) with Observations at Necropsy

Author	Date	Age of Patient	Sex	Diagnosis	Pathologic Observations	Interpretations
Hand	1893	3	M	Polyuria and tuberculosis	Yellowish granulation tissue in the cranial bone defects, liver, spleen and lymph nodes	Because lesion was not characteristic of syphilis a diagnosis of tuberculosis was made. Author did not believe the pathologic process could be attributed to tuberculosis Chronic inflammatory granulation tissue
Hochstetter and Veit	1922	44	M	Multiple sclerosis of the endocrine glands	Granulation tissue in skull, femur, pituitary gland, dura, hil of kidneys, lungs, eyelids	
Wiedman and Freeman	1924	9	M	Xanthoma tuberosum two necropsies disclosing lesions of central nervous system and other tissues	Granulation tissue containing lipoid substances found in skin, liver, lung, pleura, skull, dura, pineal gland, pituitary gland, hypothalamic region	The lesions in the liver were due to syphilis with secondary inflammatory granulation tissue
Schutz, Werbster and Puhl	1924	2	F	Granuloma like systemic disease of the hematopoietic apparatus	Granulation tissue in cranial bone defects, long bones, vertebrae, ribs, spleen, lungs, liver, lymph nodes, pancreas, myocardium	A true granuloma—chronic infectious inflammatory granulation tissue
Thompson, Keegan and Dunn	1925	9	M	Defects of membranous bones, exophthalmos and diabetes insipidus	Inflammatory granulation tissue in skull, pelvis, femurs, scapulae, clavicles, ribs, cervical and lumbar vertebrae, tuberculum, infundibulum, dura, lungs	The lesion was inflammatory or infectious rather than of degenerative or metabolic etiology
Kyrklund	1926	12	M	A rare syndrome (cranial softening, exophthalmos, adiposogenital dystrophy, diabetes insipidus)	Granulation tissue in skull, scalp, dura, hypothalamic region, kidney	A tumor of sarcomatous nature
Roland	1928	5	M	Xanthomatosis and the reticulo endothelial system	Granulation tissue containing lipoid substance found in cranial defects of skull, pituitary gland, ilium, vertebrae, lungs, thyroid gland, lymph nodes, heart, kidney	A peculiar granuloma due to deposition of lipoid substances subsequent to a metabolic disturbance
Herzenberg	1928	5	M	Niemann Pick's disease	Granulation tissue containing lipoid substances in cranial defects of skull, sternum, femurs, vertebrae, pituitary gland, infundibulum, thymus tonsils, lymph nodes, spleen, liver, skin	Chronic granuloma due to deposition of lipoid substances; constitutional anomaly causing disturbance in lipoid metabolism with formation of a chronic granuloma due to the deposition of lipoid
Schuller and Chiari	1931	29	M	Xanthomatosis	Granulation tissue containing lipoid substances in cranial defects of skull, femur, ilium, psoas, lds, pleura, lungs, hypothalamic region	Chronic inflammatory granuloma due to disturbance in lipoid metabolism
Henschen	1931	3	F	Christian syndrome	Xanthomatous changes in bone marrow of tibia, ribs, pelvis; retroperitoneal fat and loose connective tissue all over the body, spleen, lymph nodes, liver	Granulomatous lesion due to disturbance in lipoid metabolism
Ighenti	1931	3	M	General granulomatous xanthomatosis	Granulomatous xanthoma tissue in cranial defects of skull, pelvic bones, lymph nodes, liver, colon, ileum, duodenum, spleen, lungs, tonsils, tongue, skin, dura, stomach, fat about hill of kidneys	Granulomatous lesions due to disturbance in lipoid metabolism

to be as characteristic for this disease as the gumma and tubercle are for syphilis and tuberculosis.

This granuloma has three main constituents: (1) the characteristic foamy lipid cell, the specific element that contains the lipid substances; (2) the inflammatory cellular exudate, a response of the tissues to the lipid substances; (3) connective tissue proliferation.

It is the opinion of present investigators (Christian,<sup>4</sup> Chester,<sup>5</sup> Chiari,<sup>7</sup> Ighenti<sup>10</sup>) that the lesion is essentially a disturbance in lipid metabolism. To such a condition, the term xanthoma, meaning a tumor, is hardly applicable, and less so is the term xanthomatosis, meaning multiple tumors. For this reason it was suggested<sup>5</sup> that the lesion be termed "lipoidgranuloma," and the generalized form, "lipoidgranulomatosis."

#### REPORT OF CASE

*History.*—P. S., a Jew, aged 28, single, was admitted to the Neurological Division of Montefiore Hospital, May 8, 1928, complaining of (1) excessive thirst and frequent and excessive urination of eighteen months' duration, (2) a discharge from the left ear of thirteen months' duration, (3) a discharging anal fistula of ten months' duration, (4) a discharge from the right ear and a draining anus of the right thigh of four months' duration and (5) weakness and loss of weight.

The family history was irrelevant.

When the patient was 8, he had an uncomplicated scarlet fever. At 13, he suffered a fall that resulted in laceration of the face and a fracture of the nasal bone for which operative interference was necessary to relieve the nasal obstruction. At 21, he had arthritis of the ankles and knees lasting two months. Between 16 and 24 he was in good health, working as a teamster. At the latter age, he weighed 210 pounds (95.2 Kg.).

A series of symptoms and operative procedures began at 23 that were to invalid him for the remainder of his life. Because of pain an upper right molar tooth was extracted, and in this area granulation tissue appeared. All the teeth in that section, as well as many in the lower jaw, began to loosen and fall out. Within two years he had lost most of his teeth; an operation for "multilocular cyst" of the jaw was followed by a partial resection of the lower jaw for "granuloma" of the jaw. The following year polydipsia and polyuria were first noted.

At 25, he was hospitalized on several occasions for attacks of dizziness and double vision, as well as for severe pains in the left side of the chest, interpreted as "dry pleurisy." At 27, chronic eustachian salpingitis was followed by chronic otitis media in turn complicated by right mastoiditis, for which mastoidectomy was performed. Because of pain on defecation and a "yellowish" rectal discharge, he was twice operated on for fistula *in ano* and rectal abscess. Shortly thereafter he complained of severe pain in the right thigh and, as a roentgenogram revealed areas of bone absorption in the upper part of the right femur, he was operated on for osteomyelitis. About six months prior to his admission to Montefiore Hospital, a course of injections of pituitary relieved his polydipsia and polyuria considerably.

Biopsies on tissue removed from several of the sites operated on (mandible, mastoid and femur) resulted in a report of a "peculiar type of granuloma."

*Physical Examination.*—The patient was revealed as a fairly well developed adult man weighing 165 pounds (74.8 Kg.), obese despite the history of loss of weight. The skin was coarse, dry and of a pseudomyxomatous consistency. The

absence of perspiration, even in hot weather, was striking. There was a striking absence of facial hair; that of the axilla was sparse, and there was a typical female distribution of the pubic hair. Large pads of fat were present in the gluteal region.

Scars of the sites operated on were noted, with persistent draining sinuses in the mastoid, perineal and thigh areas. A purulent discharge was present in both ears, accompanied by pain in both ears and deafness in the left. The old fracture of the nasal bone and the depressed septum caused moderate nasal obstruction. The lower jaw was markedly foreshortened; most of the mandible had been resected. Only two upper teeth remained. Pyorrhea alveolaris was marked, and speech and deglutition were impaired.

There was tenderness of bones over the left side of the chest. The heart and lungs were without abnormality except for a persistent bradycardia (blood pressure, 110 systolic and 80 diastolic). The liver and spleen were not palpated. There were no abdominal masses. There was no edema of the lower extremities. Walking was difficult; genu valgum was present.

The appetite was poor, and there was habitual constipation. For the past several months the intake of fluids ranged from 6 to 12 liters, and an equivalent amount of pale watery urine was voided. At times urine was voided as frequently as every twenty minutes.

The clinical impressions at that time were: (1) pituitary dyscrasia; (2) diabetes insipidus syndrome; (3) bilateral chronic otitis media with mastoiditis; (4) chronic osteomyelitis of the right femur, probably tuberculous; (5) chronic fistula *in ano*, probably tuberculous.

*Clinical Course.*—May 21, 1928: Roentgenograms of the skull revealed an area of bone absorption in the parietal region (fig. 1 *a*) and an almost complete absence of the mandible (*b*). The sella turcica (*c*) showed no abnormalities. Roentgenograms of the right femur showed areas of bone destruction and production in the proximal portion. There was a large defect in the right wing of the sacrum. A "malignant granuloma" with metastases to the skeletal system was considered.

May 30, 1928: With administration of pituitary, 1 cc. daily, the intake of fluids was reduced from 2,700 to 3,800 cc., with a corresponding output.

June 13, 1928, to March 18, 1930: Roentgen therapy (Dr. Lenz) was begun as a palliative measure. Eventually radiation was applied to six skull fields, the right femur, the perineal field, both ears, the lower part of the left axilla, and the lower part of the right side of the chest, with a total of 23 erythema doses.

Sept. 17, 1928: The hearing in the right ear and the pain in the right parieto-temporal region were found greatly improved since the roentgen therapy.

Oct. 10, 1928: Rectal examination revealed a granulomatous perianal ulceration extending to the internal anal sphincter. A specimen of tissue taken for biopsy was reported on as chronic granulation tissue. A roentgenogram showed extension of the skull involvement.

Feb. 11, 1929: The diabetes insipidus syndrome was being well controlled with injections of pituitary. At this time it was suggested that there was involvement of Rathke's pouch with extension to the tuber cinereum.

April 13, 1929: A biopsy of the site of the previous biopsy in the axilla, which had failed to heal, showed a peculiar type of granuloma in which there were large oval cells with lipoid material in the cytoplasm.

April 20, 1929: The left external auditory meatus was completely blocked by granulations. Chronic otitis media was noted on the right side, but the hearing was improved and the discharge lessened.

June 13, 1929: There was tenderness over the ribs from the angle of the scapula to the twelfth rib in the posterior axillary line.

Aug 23, 1929. Actinomycosis or a low grade pyogenic infection was suggested as an etiologic factor. The general condition was good. The weight was 205 pounds (95 Kg.)

Sept. 19, 1929: The gain in weight and the loss of libido in a patient with dystrophia adiposogenitalis and diabetes insipidus suggested a lesion in or about the hypophysis. Perimetric examination showed concentric restriction of both visual fields plus a relatively greater limitation in the upper part of the temporal field on the left.

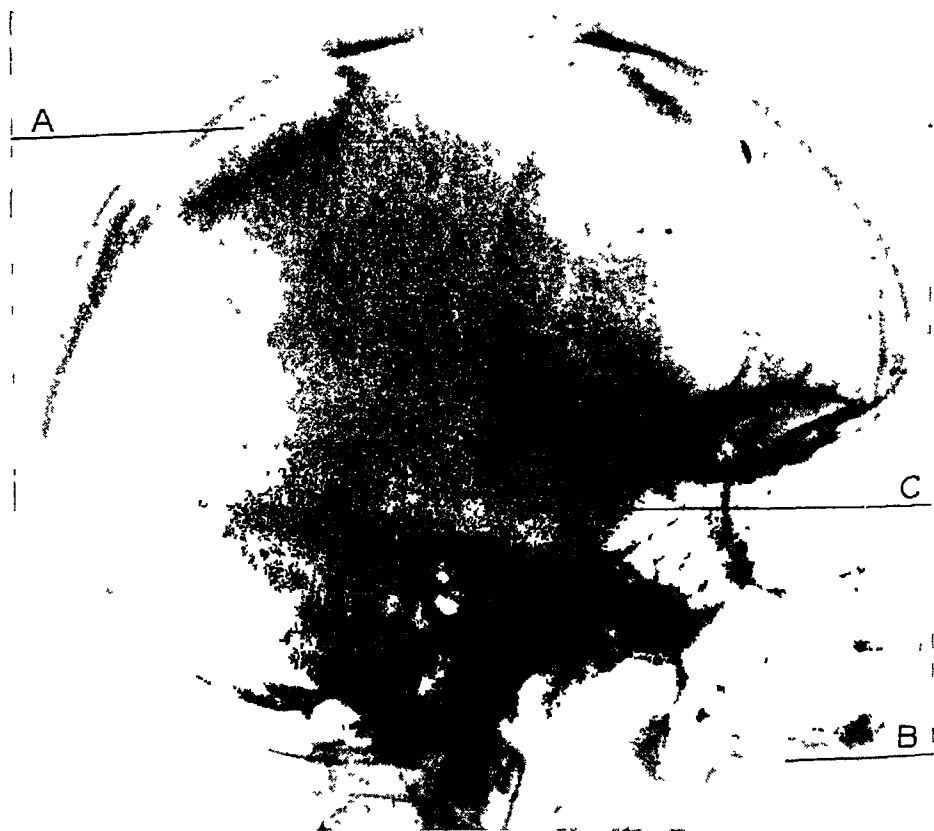


Fig. 1.—A roentgenogram of the skull showing a large defect in the parietal bone (*a*), absence of the mandible (*b*) and a normal sella turcica (*c*).

Jan. 29, 1930: A "sticking pain" was felt in the lower part of the occipital region as well as in the ankles and the right side of the chest. Nervousness, dizziness, vomiting, faintness, tachycardia, polydipsia and polyuria occurred when pituitary was not given.

May 21, 1930: Roentgen therapy had been applied to the region of the pituitary gland since Feb 20, 1930. There were noted a gain in weight (212 pounds [96.1 Kg.]), a restoration of virility, an occasional stabbing sensation in the proximal part of the right femur and pain in the chest. An occasional sinking sensation was relieved by bringing up thick, yellowish sputum. The sinuses had stopped discharging. The symptoms of diabetes insipidus were controlled with pituitary. It was deemed advisable to continue roentgen therapy.

July 7, 1930: There was cloudiness of the left antrum.

Aug. 22, 1930: The left antrum was cloudy, with an irregularity of the medial wall of the sinus, probably due to bone involvement.

Oct. 22, 1930: The edge of the liver was palpable.

Dec. 10, 1930: Progressive weakness, awkwardness, tremor of the hands and shuffling gait were noted. The patient no longer showed response to irradiation. An exploration of the pituitary gland was deemed inadvisable because of widespread metastases.

Jan. 8, 1931: The patient was discharged with the diagnosis of (1) chronic granuloma involving the jaw, skull, mastoid process, proximal end of the right femur, sacrum and perianal region, (2) diabetes insipidus and (3) Frölich's syndrome.

Jan. 13, 1931: The patient was readmitted, complaining of weakness in the knees, inability to walk, blurred vision, unsteady gait and generalized tremor. The neurologic examination showed generalized weakness, definitely more on the right than on the left, and inconstant ataxia in the upper extremities, the tremor being more marked when pituitary was withdrawn. There were also ankle clonus

TABLE 2.—*Blood Counts*

	May 13, 1928	Sept. 12, 1929	Feb. 16, 1931
Hemoglobin.....	75%	.....	62%
Platelets.....	.....	.....	450,000
Red blood cells.....	4,600,000	.....	3,100,000
White blood cells.....	8,800	10,000	10,800
Polymorphonuclears.....	72%	72%	76%
Lymphocytes.....	20%	25%	17%
Mononuclears.....	7%	....	2%
Basophils.....	1%	1%	...
Eosinophils.....	...	2%	5%

on the right, an equivocal Babinski sign, Hoffmann's sign on the right, and concentric restriction of the visual fields. There were sensory disturbances in the left trigeminal area, which might have been due to an old peripheral lesion of the facial nerve or to involvement of the gasserian ganglion. The neurologic consultants thought that these findings indicated an extension of the pathologic process to the adjacent portions of the cerebral hemisphere on the left.

Jan. 31, 1931: The diagnosis of Hand's disease (Schüller-Christian syndrome) was made. It was thought that the entire clinical picture could be explained by lipoidgranulomatous infiltrations of the hypophysis and the hypothalamic region, causing dystrophia adiposogenitalis, diabetes insipidus and extensive osteoclastic skeletal changes with involvement of the skull, alveolar process of the maxillary bone, mandible, mastoid process, proximal end of the right femur, sacrum and ninth rib on the left.

Feb. 8, 1931: An osteoclastic defect was revealed in the proximal portion of the left tibia.

March 3, 1931: The patient's condition had become progressively worse. The profound asthenia was unusually striking. He died on March 4, 1931, of what was considered to be hypostatic pneumonia.

*Laboratory Data.*—The blood counts are shown in table 2. The bleeding time was  $5\frac{1}{4}$  minutes; the coagulation time,  $9\frac{2}{3}$  minutes; the



clot reaction time, 3 hours. The result of the tourniquet test was negative. The blood icteric index was 6. The result of the van den Bergh test was normal. In a congo red test, 85 per cent of the dye was retained in the blood stream after one hour. The blood chemistry was as follows: urea nitrogen, 7.7 mg. per hundred cubic centimeters; cholesterol, 152 mg.; calcium, 9.8 mg.; phosphorus, 3.9 mg.; total fat, 1.71 per cent; serum proteins, 7.49 per cent; albumin, 4.71 per cent, and globulin, 3.23 per cent.

The Wassermann test of the blood and that of the spinal fluid gave negative results. The spinal fluid was normal. The spinal fluid fat was 12 mg. per hundred cubic centimeters; the protein, 42.8 mg. The sputum showed no tubercle bacilli or other micro-organisms. The urine was normal and showed no Bence-Jones protein. A complement-fixation test for *B. mallei* was negative.

A sugar tolerance test on Jan. 16, 1931, yielded results as follows: fasting, 89 mg. per hundred cubic centimeters; one hour after the administration of 100 Gm. of dextrose, 110 mg.; two hours after, 86 mg. A test made on Nov. 15, 1929, gave the following results: fasting, 87 mg.; one-half hour after the administration of 100 Gm. of dextrose, 114 mg.; one hour after, 77 mg., and two hours after, 79 mg.

The basal metabolism was plus 1 per cent in the first determination and minus 4 per cent in the second. An electrocardiogram showed left axis deviation and sinus bradycardia.

*Autopsy* (Dr. David Perla).—The body was that of an obese young man, the general configuration, deposition of fat tissue and distribution of pelvic hair as in dystrophia adiposogenitalis. The skin was smooth, dry and pale. On the face was a linear scar extending from the left nostril to the frontal bone in the midline. The lower half of the mandible was absent, and there was a deep transverse scar across the chin. Only two of the upper teeth were present; the contiguous tissue was pale and yellowish on section. Deep scars were present in the left axilla and over the proximal portion of the right femur. There was pitting edema of the lower extremities.

Significant changes were present in the lungs, pituitary gland, osseous system and testicles.

**Lungs:** The lungs were grayish red and contained air throughout. The pleura was slightly thickened. Besides small patches of bronchopneumonia in both lower lobes, the cut surface showed numerous small, irregular yellow nodules varying from 0.5 to 2 cm. in size. Within these areas were small yellow spots from which grayish strands extended into the surrounding tissue. The pulmonary vessels appeared normal. The bronchi were congested.

**Pituitary Gland:** The pituitary gland was slightly enlarged, and on section was grayish white. The capsule was thickened.

**Osseous System:** The calvarium showed a large punched-out area about the size of a small plum in the midportion of the parietal bone (fig. 1a) extending from both sides of the longitudinal suture. The cortex was eroded, and both tables

were replaced by a dense membrane. The region of the sella turcica, the sphenoidal sinuses and the base of the skull showed no abnormalities. The frontal bones and the retro-orbital regions were normal.

The cut surface of the proximal portion of the right femur showed the cortex to be markedly thickened, almost entirely obliterating the medullary cavity for a distance of 3.5 cm. The medulla was there replaced by a grayish-white sclerosed tissue of the consistency of bone. Distal to this area, the medullary cavity contained fat marrow.

Several of the lumbar and thoracic vertebrae and ribs showed no gross abnormalities.

Testes: The testes were atrophic and on section consisted of dense fibrous tissue.

Other Organs: The thyroid gland showed a moderate amount of colloid and no abnormalities. The pancreas was firm and grayish yellow; it revealed no abnormalities. The right suprarenal gland weighed 9.5 Gm.; the left, 14.5 Gm. The cortex was narrow and contained the usual amount of lipoid. The medulla showed moderate postmortem autolysis. The heart weighed 440 Gm. It showed "tigerling" of the right ventricle. The liver, spleen and lymph nodes showed no significant gross changes. These as well as the other organs are mentioned in the microscopic protocol.

*Microscopic Observations.*—The pleura was thickened as were the interlobular and alveolar septums, the latter often showing marked cellular infiltration. Numerous areas were edematous and congested and the alveoli filled with a conglomerate of large, vacuolated cells. These appeared to be proliferated, highly vacuolated, alveolar epithelial cells and were apparently different morphologically from the lipoid elements in the granulomatous tissue in other organs. The same plugs were seen in some of the smaller bronchi, about which there was some connective tissue proliferation with little cellular infiltration. With sudan III, these vacuolated areas stained from yellowish red to brown, very little of the sudanophilic substance refracting polarized light. With Smith-Dietrich stain there was little bluish-staining substance in this area. In some areas, pulmonary tissue was barely recognizable and was replaced by a granulomatous tissue consisting of fibrous connective tissue elements and a considerable number of lymphocytes and a few plasma cells. In a few areas there was an increase in perivascular connective tissue.

Pituitary Gland: The capsule was considerably thickened. At the junction of the lateral posterior portion of the anterior lobe and the posterior lobe was a large, fairly circumscribed nodule consisting of large, closely packed, polyhedral cells, lying in a meshwork of connective tissue fibers. These cells (fig. 2) had small, oval or round, darkly staining nuclei, rich in chromatin. Occasionally, vesicular reniform, and bizarre, irregularly formed nuclei were seen. With Mallory's trianiline stain the nuclei stained red. The cytoplasm was plentiful and honeycombed or foamy in appearance; occasionally, however, vacuolated forms were seen. The cell membranes were usually distinct, though occasionally rather indefinite because of the compactness of these cells. These lipoid cells (*Schaumzellen*, xanthoma cells) were also seen in isolated groups and even singly. A few fat cells were also seen in this nodule. The connective tissue proliferation (fig. 2f) seemed to be in inverse ratio to the number of lipoid cells, so that in some areas the connective tissue elements and round cell infiltration predominated, and only an occasional lipoid cell was seen. In some areas the lipoid cells were absent and apparently replaced by dense, fibrous, fairly acellular connective tissue.

The anterior lobe (fig. 3) was at least twice normal size. The vessels were considerably dilated and filled to various degrees with cellular elements of the blood. The stroma (fig. 3 *g*) showed two small, fairly dense areas of connective tissue fibers in the lateral portion of the anterior lobe, one on each side, containing numerous fibroblasts, a few small blood vessels distended with cellular elements, and groups of basophils in alveolar structure, in the centers of which were occasional chromophobe cells with greatly increased protoplasmic structure and large vesicular nuclei. There were also small areas of hemorrhage in these scarlike areas of connective tissue. The connective tissue gradually tapered out and became continuous with the rest of the stroma of the gland.

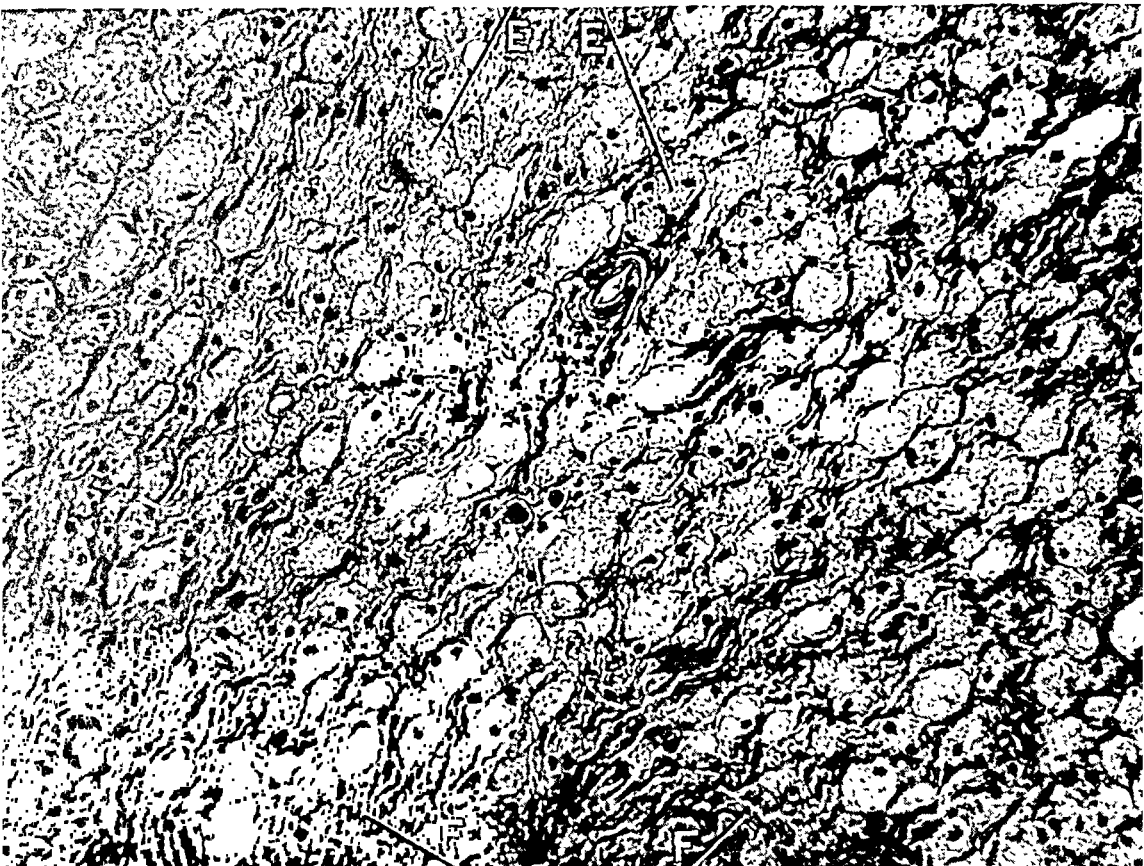


Fig. 2.—A lipid granuloma showing lipid cells (*e*) and connective tissue proliferation (*f*).

The alveolar structure was preserved throughout. The eosinophils were greatly reduced in number and were irregularly and diffusely scattered throughout the anterior lobe, being found in greater numbers in the posterior portion. The nuclei were often eccentric and varied considerably in size. The protoplasm contained numerous coarse and fine eosinophilic granules. The cell membranes were distinct. The basophils, although most numerous in the anterior portion of the anterior lobe, were also found in large numbers throughout the rest of the lobe, as well as in large groups in the tissues between the anterior and posterior lobes. The basophilic foci were composed either of single alveoli or of groups of alveoli in which practically all the cells were of the large, heavily granulated, deeply staining basophilic

type (fig. 3 *h*). Smaller varieties were seen in the so-called "pars intermedia." In the basophilic areas a distinct thickening of the stroma was noted. The nuclei were oval or slightly irregular and often eccentric and varied considerably in size. The cell membranes were distinct, the cellular configuration depending on the massing of the cells.

Diffusely throughout the anterior lobe, particularly in the lateral, posterior and central portions, either singly or in groups, at times forming complete alveoli, were numerous altered chromophobe cells (fig. 3 *i*).

Occasionally, these cells were found in the center of an alveolus, the common sites of their precursors, the main cells, surrounded by basophils and eosinophils.

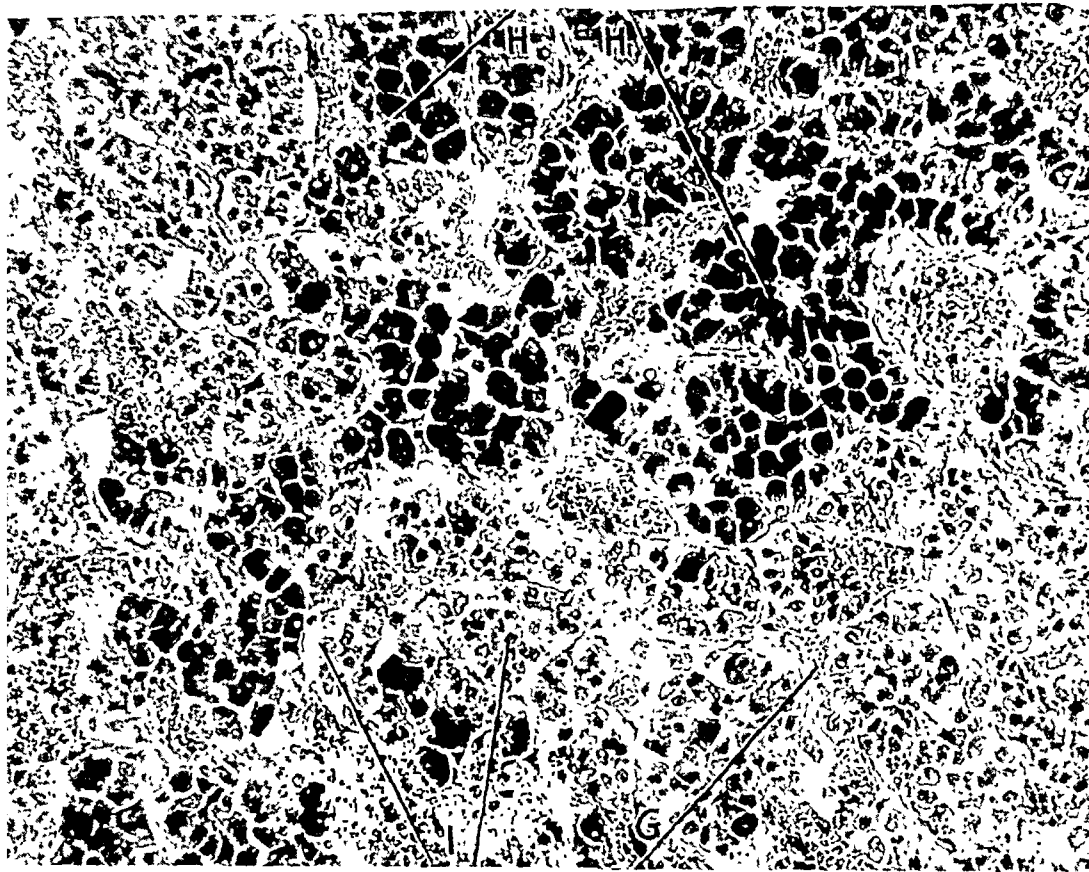


Fig. 3.—A section of the anterior lobe showing thickened stroma (*g*), alveoli formed of large basophils (*h*) and alveoli formed mainly of altered chromophobe cells (*i*).

The nuclei varied in size; they were round or oval, vesicular, and occasionally irregular in form with varying chromatin content. The protoplasm stained pale brown (hematoxylin and eosin), varied in amount, and was granular. There was no distinct cellular membrane; the nuclei often appeared to lie in small masses of protoplasm. The protoplasm stained faintly reddish with the hematoxylin and eosin and Mallory trianiline stains.<sup>13</sup> Though too numerous to count, the altered

13. These cells have been termed "pregnancy cells" (*Schwangerschaftszellen*) by Erdheim and Stumme (*Beitr. z. path. Anat. u. z. allg. Path.* 46:1, 1909).

chromophobe cells comprised at least 50 per cent of the cells of the anterior lobe. Some of these contained isotropic fat globules. Occasionally, round cell infiltrations were seen in the posterior lobe in the region of the lipoidgranuloma. The epithelial investment of the posterior lobe was particularly rich in basophils. In the intervening tissue between the anterior and posterior lobes termed by some "pars intermedia" were islands or groups of cells arranged around cavities filled with dense colloid material and lined with cuboidal epithelium (retention cysts resembling greatly the alveoli of the thyroid gland). Within the colloid substance were desquamated epithelial cells. In the posterolateral portion of the anterior lobe were several large cysts, which were lined with squamous, columnar, occasionally ciliated epithelium, and which contained a gelatinous substance (vestiges of the hypophyseal cavity).

Occasionally, plasma cells were seen. Isotropic droplets were seen throughout the epithelial elements of the anterior lobe, perhaps most marked in the basophils.

The posterior lobe consisted of dense, fairly acellular bands of matted together connective tissue fibers. The neuroglial elements were closely compressed; the interneuroglial spaces were practically obliterated. The entire lobe appeared atrophic and fibrotic, and there was a marked round cell infiltration.

**Osseous System:** Sections from the region of the cranial bone defect showed partial replacement of both tables by dense, acellular connective tissue which filled the diploe. The contiguous trabeculae showed arrested development. The osteoblasts were flat throughout. There was considerable evidence of bone destruction, namely, eroded trabeculae, in the vicinity of which were numerous osteoclasts. The dense connective tissue, which contained some blue-staining areas, probably calcified fibrous tissue, extended in places to the periosteum. Distal to the lesion there also appeared to be arrested development of the trabeculae.

Sections from the proximal portion of the right femur showed fibrous replacement of the spongiosa, so that the entire area involved resembled compact bone. The fibrous tissue extended between the trabeculae, often enclosing them entirely. In some areas there was considerable osteoblastic proliferation, the osteoid tissue being quite thick. In other areas osteoclastic changes predominated. A few of the haversian canals were dilated; the regional bone was eroded and contained lipid cells. The predominant feature in the femur was the connective tissue replacement of the spongiosa by an osteosclerotic process.

Sections from the region of the sella turcica, ribs and vertebrae showed no significant changes.

**Testes:** These presented marked atrophy of the seminiferous tubules bilaterally, more marked on the left, with replacement fibrosis and hyalinization. The interstitial cells were greatly decreased in number. The remaining intact tubules showed complete loss of spermatogenesis.

**Other Organs:** The pineal body showed some hyalinization and connective tissue fibrosis with slight calcification.

The thyroid gland showed the follicles distended with colloid substances. The lining epithelium was flat. No lipid cells were seen.

The pancreas showed slight autolysis. The islands of Langerhans were numerous, large and prominent. The nuclei of the epithelial cells were large and vesicular.

In the suprarenal glands, the medulla was unusually prominent and contained two definite ganglionic masses. The muscle coats of the suprarenal veins were irregularly hypertrophied. The cortex was thin. There was marked atrophy of the fascicular layer. The glomerular layer contained a moderate amount of lipid substance.

The germinal centers of the lymph nodes were not distinct. There were several areas of hyalinization. The sinuses were filled with proliferated endothelial cells. No lipoid cells were seen.

In the spleen, the malpighian corpuscles were prominent. Small scattered areas of hemorrhage were present in the pulp. The sinuses were obscured; there was a slight increase in the endothelial elements. The walls of the smaller arterioles were thickened. There were no lipoid cells present.

The kidneys showed a moderate amount of parenchymatous degeneration. The glomeruli were normal.

In the prostate, some glands showed occasional epithelial hyperplasia. One gland was filled with proliferated epithelial cells.

The epididymis showed increased density of the connective tissue fibers between the tubules.

The gums showed cellular infiltration, consisting of plasma cells, lymphocytes and enlarged cells with vesicular nuclei. No lipoid cells were present.

The liver showed parenchymatous degeneration.

The tongue, esophagus, stomach, small and large intestines, gallbladder, diaphragm, heart, aorta, pulmonary artery, trachea, skin, and axillary and abdominal fat showed no significant abnormalities.

#### COMMENT

*Age.*—Hand's disease (Schüller-Christian syndrome) is seen most frequently in children, thirty-three of the fifty reported cases having occurred in children in the first decade of life. The youngest was 2 years old, and 50 per cent were less than 5 years old. Cases were reported as occurring in persons as late as the sixth decade, the oldest of these persons being 55 years.

No.	26	7	6	6	2	2	1	
Age	0	5	10	20	30	40	50	60

*Sex.*—Males are predominantly affected in about the proportion of 2:1. Of fifty cases, thirty-four occurred in males and sixteen in females.

*Etiology.*—The etiology of this disease is unknown. In several instances lipemia with hypercholesteremia has been noted. Normal or even subnormal values of the fat and lipoid in the blood stream, however, are not incompatible with the existence of this disease. There is neither a climatic nor a geographic influence. Similar to other types of disturbances in lipid metabolism (Niemann-Pick's disease and Gaucher's disease) it is particularly frequent among Jews. As the disease appears early in childhood, it is evident that occupation plays no important rôle. Frequently trauma appears to be the precipitating factor. According to the literature, heredity is not a factor, although there is one questionable instance of two cases occurring in one family (Herzenberg).

*Symptomatology.*—The sites of predilection for the deposition of the lipid substances are the tissues of the head region, the structures being involved in the following order: (1) cranial bones, (2) orbit and (3) region of the pituitary gland and the tuber cinereum. The three cardinal symptoms of Hand's disease, (1) defects of the cranial bones, (2) exophthalmos and (3) diabetes insipidus, are directly referable to the lipidgranulomatous deposits in these regions.

1. The skeletal system is particularly prone to lipidgranulomatous involvement. The predominant osteoclastic changes in the cranial bones give rise to the palpable, often pulsating and roentgenologically demonstrable, cranial bone defects. The roentgenogram of the skull is characteristic, giving rise to the *landkarten Schädel* ("map skull") (Schüller). The process is not merely confined to the cranial bones, but may also involve other parts of the skeleton, namely, the humeri, femurs, scapulae, ribs, vertebrae and pelvic bones (Thompson, Keegan and Dunn; Hochstetter and Veit; Schultz, Werbter and Puhl; Rowland; Herzenberg, Schüller and Chiari; Sosman; Ighenti, and others). In the case reported in this communication, the sacrum, femur and tibia were also involved. There was also cloudiness of the left antrum of Highmore with irregularity of the medial wall due to bony involvement. Thompson, Keegan and Dunn and Herzenberg also reported sinus involvement. The severe skeletal pains are subsequent to the lipidgranulomatous changes and are particularly marked when the lesions are near the periosteum.

2. The exophthalmos, which is sometimes unilateral (Hand, case 3; Schüller and Chiari) and exceptionally absent (Globig; Herzenberg; Sosman; our case), is due to the destruction of the orbital plate of the frontal bone by the granulomatous process, which at times may involve the retro-orbital structures (Rowland, case 1; Schüller and Chiari; Wheeler). Lipidgranulomatous involvement of the sympathetic fibers to the müllerian muscle may also be a factor in the causation of the proptosis.

3. The diabetes insipidus syndrome is referable to lipidgranulomatous involvement of either the pituitary gland or the region of the tuber cinereum. Occasionally, as in our case, both of these structures show extensive involvement. Roentgenograms of the region of the sella turcica may show extensive destruction of the floor of the sella turcica and clinoid processes (Christian; Rowland, case 2; Cohen, Moreau and Murdoch; Pickham and Joel). On the other hand, this may be perfectly normal (Grosh and Steffel; Hand, case 2; Hausmann and Bromberg; our case, and others).

Besides the cardinal symptoms forming the diagnostic triad of defects in the cranial bones, exophthalmos and diabetes insipidus, there are inconstant, though very significant other clinical findings in Hand's disease, as follows:

1. Gingivitis occurs with painful gums and falling out of the teeth due to lipidgranulomatous destruction of the alveolar processes of the maxillary bones, though this may be influenced as well by the lesion in the pituitary gland. These symptoms are frequently present very early in the disease, and it is not uncommon for the dentist to note the presence of a peculiar yellowish granulation tissue in the socket of an extracted tooth or about the gum of a loose tooth.

2. Endocrine dyscrasias are found. Of these, the most common is dystrophia adiposogenitalis (Fröhlich's syndrome) as in the instances reported by Schüller, Kyrklund, and Schüller and Chiari. Dwarfism was noted by Grosh and Steffel, Alberti, Schultz, Wermbter and Puhl, and Rowland. Simond's cachexia was reported by Hochstetter and Veit in a case with a pluriglandular syndrome. The asthenia is often unusually marked. Mental and physical retardation are mentioned by many authors. Our case showed a pluriglandular picture with predominant features of dyspituitarism.

3. Splenohepatomegaly was noted by Herzenberg, Schultz, Wermbter and Puhl, and Ighenti. Wiedman and Freeman noted icterus associated with hepatomegaly due to obstruction of the portal passages by involved glands.

4. Lymphadenopathy was noted by Berkheiser, Vampré, Herzenberg, Ighenti, and Kartagener and Fischer.<sup>14</sup> The barely palpable axillary lymph nodes in our case showed lipoid cells on biopsy.

5. The neurologic manifestations of this disease are varied, depending on the localization of the granulomatous lesions. Thus signs of increased intracranial pressure are mentioned by Hochstetter and Veit, Schüller and Chiari, and Frummann-Dahl and Forsberg. In the case of Schüller and Chiari, focalizing signs of intracranial tumor were present as well. Signs referable to diffuse involvement of the central nervous system were present in our case. This aspect will be reported in a subsequent publication from the Neuropathological Department by Dr. Charles Davison.

6. Cutaneous lesions were noted by Hand, Herzenberg, Höfer and Ighenti. These may vary from bronzing to maculopustular, hemorrhagic or seborrhea-like lesions. Lipoid infiltrations of the eyelids (*Dachshund-Augen*) have been reported by Schüller and Chiari, Hochstetter and Veit, Pussey and Johnstone, and Kartagener and Fischer.

7. Transient impotence was present in our case as well as in the instance reported by Hochstetter and Veit.

8. Disturbances in hearing are mentioned by Höfer. Ighenti's patient had an external otitis. Turner, Davidson and White reported involvement of the mucous membrane of the mouth, larynx, trachea and bronchi. In this case, tracheotomy was performed because of laryngeal obstruction by a lipoid granulomatous mass.

9. Cor pulmonale with failure of the right side of the heart was reported by Thompson, Keegan and Dunn. Chester reported cor pulmonale due to collapse induration of the lungs subsequent to lipoidgranulomatous infiltrations in a type of lipoidgranulomatosis confined to the skeletal system (not in the cranial bones) and the internal organs.

10. Susceptibility to infection at the site of the local lesion as well as to intercurrent often terminating infections is a characteristic feature. In our case, the formation of persistent draining sinuses at the areas operated on is noteworthy.

Because of the widespread lesions and the subsequent protean clinical manifestations, a differential diagnosis offers considerable difficulty. A review of our case alone illustrates this contention. The diagnoses considered were the following: (1) various types of neoplasm with skeletal metastases, such as Ewing's sarcoma, sarcoma of the reticulo-

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14. Kartagener, M., and Fischer, H.: Ztschr. f. klin. Med. **119**:421, 1932.



endothelial system, primary pituitary neoplasms, multiple myeloma; (2) Hodgkin's disease; (3) various types of granulomas, such as syphilis, tuberculosis, actinomycosis, glanders; (4) chronic nonspecific granuloma.

Apart from the presented classic picture of Hand's disease and the unusually extensive involvement of the central nervous system, this case is particularly interesting because of its endocrinologic aspects. The patient presents the clinical picture of dyspituitarism, namely, marked skeletal overgrowth, long arms, thick wrists, big feet, adiposity, wide pelvis, female distribution of pelvic hair, gluteal pads of fat, coarse, pseudomyxomatous, waxlike color of the skin, hypotrichosis of the face and lower extremities, asthenia, drowsiness, bradycardia, low blood pressure, subnormal temperature, lack of perspiration plus diabetes insipidus and loss of libido. The enlargement of the anterior lobe of the pituitary gland associated with a marked diminution of the number of eosinophils, a definite hyperplasia and hypertrophy of basophils, an increase in the stroma, presence of a lipoidgranuloma in the capsule, a peculiar transformation of the chromophobe cells and marked fibrosis, atrophy and round cell infiltration of the posterior lobe is striking. The hyperplasia and hypertrophy of the basophils are noteworthy. It is of interest to note that Cushing, at a recent meeting of the Section of Neurology of the New York Academy of Medicine, suggested the possibility of an unusual clinical syndrome associated with basophilic adenoma of the pituitary gland. Kraus<sup>15</sup> attempted to draw a relationship between constitution and the basophilic content of the anterior lobe of the pituitary gland. Designating a patient of normal proportions as mesothenic, he denoted the basophilic content of the anterior lobe as 3 plus. Asthenic persons, as those with tuberculosis or Addison's disease, have a greatly diminished number of basophils, while hyperasthenic and obese patients have a greatly increased basophilic content. Thus the habitus in this case may be a factor related to the unusual number of basophils. The pituitary struma by pressure on the optic chiasma was possibly responsible for the marked diminution of vision and temporal notching of the visual field, both of which had increased in degree at the second admission to the hospital. Reuse<sup>16</sup> noted transient bitemporal hemianopia during pregnancy, which disappeared after parturition.

There is a definite reciprocal relationship between the pituitary gland and the testes. The association of lesions of the pituitary gland with subsequent gonadal atrophy has been noted by Custriny,<sup>17</sup> Bar-

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15. Kraus, E. J.: *Virchows Arch. f. path. Anat.* **268**:315, 1928.

16. Reuse, cited by Erdheim, J., and Stumme, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **46**:1, 1909.

17. Cited by Kon, J.: *Beitr. z. path. Anat. u. z. allg. Path.* **44**:233, 1908.

tels,<sup>18</sup> Cushing<sup>19</sup> and Biedel.<sup>18</sup> It is of interest to note, however, that Tandler and Gross<sup>20</sup> reported roentgen changes in the sella turcica in eunuchs. In the instance reported in the present communication, we believe that the changes in the pituitary gland are primary and testicular atrophy secondary for the following reasons:

1. In women following roentgen castration, Kon,<sup>21</sup> Kolde<sup>22</sup> and Rössle<sup>22</sup> found engorgement of the pituitary gland. In these instances, marked hyperplasia and hypertrophy of the eosinophils and striking diminution of the basophils were noted. In a case of absence of the ovaries, Olivet<sup>22</sup> reported a large pituitary gland showing a marked increase in the eosinophils and a small nodule of main cells.

In eunuchs, the increase in the weight of the pituitary gland as shown by Fischerer<sup>17</sup> is due to a marked increase in the eosinophils. Only in rare instances, notably the cases of Garfunkel<sup>23</sup> and Kon,<sup>21</sup> were the findings different. In these there was a diminution in the eosinophils and basophils, and the chromophobe cells showed the changes associated with pregnancy, namely, the appearance of "pregnancy cells."

2. In cases of dyspituitarism of the Frölich type, the testes may show no abnormality.

3. The early symptoms of dyspituitarism in this case, as already noted, were present years before the impairment of libido or of potency.

In view of the gonadal atrophy it is significant to note the prominence of the islands of Langerhans in the pancreas. Gottlieb<sup>22</sup> and Rössle<sup>22</sup> reported hypertrophy of these islands after loss of testicular function.

In the differential diagnosis the frequent confusion with multiple myeloma is striking. In Gilmore's case of Hand's disease,<sup>24</sup> Bence-Jones protein was found in the urine, making the diagnosis even more difficult. In view of the skeletal changes and the fact that the Bence-Jones albuminose is present in other conditions even when there is no skeletal involvement, Bence-Jones albuminose should be looked for in every case of Hand's disease. Tumors with skeletal metastases as carcinomas of the prostate, thyroid gland, bronchus, breast, etc., as well as hypernephroma, should also be excluded.

The "peculiar yellow" sputum in this disease, provided it is not due to fatty degeneration of a pneumonic process, may be of value in detecting pulmonary involvement in the absence of roentgen findings.

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18. Cited by Garfunkel, B.: *Beitr. z. path. Anat. u. z. allg. Path.* **72**:475, 1924.

19. Cushing, H.: *The Pituitary Body and Its Disorders*, Philadelphia, J. B. Lippincott Company, 1912.

20. Tandler and Gross: *Wien. klin. Wchnschr.* **21**:277, 1908.

21. Kon, J.: *Beitr. z. path. Anat. u. z. allg. Path.* **44**:233, 1908.

22. Cited by Kraus, E. J.: *Drüsen mit innerer Sekretion*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 8.

23. Garfunkel, B.: *Beitr. z. path. Anat. u. z. allg. Path.* **72**:475, 1924.

24. Gilmore, M. E.: *Texas State J. Med.* **21**:358, 1925.

The relief from some of the subjective symptoms at the time the patient was receiving roentgen therapy is significant. Schüller first noted the symptomatic and roentgenologic improvement in the skeletal lesions with roentgen therapy. It is very likely that the irradiation accelerates the healing process, which of itself goes on to fibrosis. Spontaneous remissions, however, occur, and progressive lesions may be detected during the course of roentgen therapy (our case and numerous others). A diet in which fat is low and various endocrine products (thyroid, parathyroid, pituitary, insulin) have been tried without success.

The evidence is insufficient for an attempted correlation of the unusual changes in the pituitary gland with this generalized disturbance of lipid metabolism. It is apparent that the changes in the pituitary gland are not subsequent to roentgen therapy.

#### SUMMARY <sup>25</sup>

A case of lipoidgranulomatosis (type, Hand-Schüller-Christian) in a man of 31 years with diffuse skeletal involvement, diabetes insipidus, dyspituitarism and extensive changes in the central nervous system is described.

Postmortem examination showed (*a*) lipoidgranulomatous lesions in the skull, femur, pituitary gland and lungs and (*b*) increased vascularity in the anterior lobe of the pituitary gland, marked diminution in the number of the eosinophilic cells, adenomatous hyperplasia and hypertrophy of the basophilic cells, a predominance of altered chromophobe cells and an increase in the stroma. The posterior lobe showed an increase in the stroma, marked fibrosis and round cell infiltration.

The atrophy of the testicles, with its attendant clinical picture, was subsequent to a primary lesion of the pituitary gland.

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25. For the sake of brevity we have listed in the footnotes only the most comprehensive bibliographic articles. Detailed references to all the cases mentioned may be found in the publications cited in footnotes 1 to 9 and 14 and 24. A complete review of the literature may be found in the publications cited in footnotes 4 to 10.

# MECHANISM OF CALCIFICATION IN THE HEART AND AORTA IN HYPERVITAMINOSIS D

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Many confusing elements may be found in the explanations that pertain to the action of vitamin D, particularly with regard to the toxic effect which may be observed when experimental animals are given enormous doses of irradiated ergosterol. First, there has been some controversy as to whether the toxic effects were the result of the action of the vitamin or of that of other substances in the preparations used, but recent work, particularly that of Harris and Moore,<sup>1</sup> and that of Harris and Innes,<sup>2</sup> appears to demonstrate that, in sufficiently large doses, the vitamin per se is toxic. Second, there is the problem whether the vitamin may not have a dual action, its beneficial effects in the treatment of rickets representing a different sort of activity from that observed in toxic hypervitaminosis. Third, there is the problem of whether, both in therapeutic and toxic doses, it has a local action on tissue or whether its action is only a general one on the calcium metabolism. Fourth, there is still a difference of opinion concerning its manner of action, some thinking it increases the solubility of calcium in the blood, and others that it acts through the agency of the parathyroid gland or of its hormone.

It would seem that a solution of the problem concerning the mechanism of calcification in hypervitaminosis D would be of no small assistance in understanding some phases of the action of the vitamin. Many investigators have reported on the appearance of changes in the recipient tissues which are preliminary to the calcifications, and Vanderveer<sup>3</sup> in a recent article depicted the results of careful histologic study in this field. Most of the experiments of this nature, however, have been conducted by the administration of the ergosterol over a relatively long period of time, so that there are difficulties in the way of deciding on the early changes. In this respect, the method used by Laas<sup>4</sup> has much to commend it, as he administered the substance in single massive doses, and found that there was a latent period of only four days before calcifica-

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1. Harris, L. J., and Moore, T.: *Biochem. J.* **22**:1461, 1928; **23**:261, 1929.

2. Harris, L. J., and Innes, J. R. M.: *Biochem. J.* **25**:367, 1931.

3. Vanderveer, H. L.: *Arch. Path.* **12**:941, 1931.

4. Laas, E.: *Virchows Arch. f. path. Anat.* **278**:346, 1930.

tions occurred. In any event, there is at this time a considerable amount of opinion that leans to the point of view that in hypervitaminosis D there are evidences of degeneration in the tissues before calcifications occur.

A decision as to the sequence of events in the tissues demonstrating calcification is of utmost importance, because it would throw a great deal of light on the question whether the action of the toxic doses is a local injurious one on tissues or only a general one on the calcium metabolism. In other words, it is important to decide whether the calcifications are the result of degenerative changes in the tissues or of a precipitation of calcium from the blood. In this connection, pathologic calcifications may be divided into two broad groups. The first group depends for causation on the degenerative changes in the recipient tissues, and the mechanism may be either that described by Klotz,<sup>5</sup> which depends on the breaking-down of fat with the formation of soaps and later more permanent deposits of calcium, or that of Wells,<sup>6</sup> which depends on the tissues assuming characteristics more physical than chemical, which enable them to bind calcium. The second group of pathologic calcifications, are, however, fundamentally different, so far as their etiology is concerned. They depend for their causation, not on degenerative changes in the recipient tissues, but on a change in the blood, so that it becomes unable to retain all its calcium in solution. A good example of calcifications that depend on this second type of mechanism may be found in the metastatic calcifications seen in hypercalcemias associated with certain parathyroid tumors.

This work is, therefore, concerned with a study of the sequence of events in the tissues in order that the calcifications may be classified. It was thought advisable to produce the lesions not only by long continued administration of vitamin D but also by administration of massive single doses, so that the cycle of events could be clearly followed. In one series, animals were killed each twenty-four hours after a large single dose of activated ergosterol.<sup>7</sup> One other point regarding the experimental procedure is of interest, namely, the method by which calcium was demonstrated in the tissues. Since it is evident from the work of Cameron<sup>8</sup> that neither hematoxylin nor silver preparations are specific

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5. Klotz, O.: *J. Exper. Med.* **7**:633, 1905; **8**:322 and 504, 1906.

6. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

7. In this paper the term "activated ergosterol" refers to irradiated ergosterol of unusual potency. The term "viosterol" is used in this paper as the substance commonly employed in therapeutic procedure. Strictly speaking, viosterol is a designation for irradiated ergosterol, but in the sense used in this paper it applies only to the strengths as found in the commercial preparations.

8. Cameron, J. R.: *J. Path. & Bact.* **33**:929, 1930.

stains for calcium, it was thought that the technic of incineration, as used by Policard,<sup>9</sup> Policard and Okkels<sup>10</sup> and Scott<sup>11</sup> for the detection of mineral content in microscopic sections, might prove to be the best method for demonstrating any increase in mineral content in the tissues. Duplicate sections of the stained preparations were prepared throughout the work by this technic.

#### MATERIAL AND METHODS

Forty rats of good laboratory stock were used in the experiments. These were divided into four series.

Series 1 consisted of seven experimental animals and seven controls. The animals were full-grown males. Each received approximately 3 cc. of viosterol, 250 D, per day. One experimental animal and one control were killed each fifth day.

Series 2 consisted of five rats, each of which was given 3 cc. of viosterol, 250 D, each day. They were killed at various times ranging from twenty-eight to thirty-eight days.

Series 3 consisted of seven experimental and four control young animals. Each of the former received 0.33 cc. of activated ergosterol 10,000 X, at the beginning of the experiment. One rat was killed at the end of forty-eight hours, and on the fifth morning the remaining six rats were found dead in the cage.

Series 4 consisted of ten half-grown male rats. Each of these received 0.25 cc. of activated ergosterol 10,000 X at the beginning of the experiment, and two rats were killed each twenty-four hours following the single administration.

The vitamin was administered per os by a dropper in series 1, and by mixing it with the food in the other three series. The animals were on a stock laboratory diet which contained an ample supply of calcium. The animals were in every case killed by ether anesthesia. Three animals in series two were given an injection of alizarin red, 2 cc. of a 1 per cent solution. Material taken at autopsy included the heart, aorta, lungs, spleen, liver, kidneys, thyroid and parathyroid glands, long bones and incisor teeth. Specimens were fixed in formaldehyde, Flemming's solution and alcohol formaldehyde. Some frozen sections were cut and stained for fat with scarlet red. Paraffin sections of the complete series were stained with hematoxylin and eosin. Other sections were stained with alizarin and with osmic acid and safranin. A complete set of serial sections duplicating the hematoxylin and eosin series was prepared for incineration by the following technic: The fixative used was 9 parts absolute alcohol and 1 part neutral formaldehyde. Blocks were then moved directly into absolute alcohol and embedded by the usual method in paraffin. Sections were then cut and floated on slides with paraffin oil. Sections were then incinerated for ten minutes at approximately 178 C., five minutes at approximately 256 C., five minutes at approximately 350 C., five minutes at approximately 454 C., five minutes at approximately 556 C., and a minute and a half at approximately 600 C. They were examined by dark field illumination.

9. Policard, A.: *Protoplasma* 7:464, 1929.

10. Policard, A., and Okkels, H.: *Anat. Rec.* 44:349, 1930.

11. Scott, G. H.: *Bull. d'histol. appliq. à la physiol.* 7:251, 1930.

## OBSERVATIONS

In series 1, each experimental animal received daily 3 cc. of viosterol and an animal was killed about every fifth day. The aortas showed little change until the seventh rat was killed, after thirty-nine days of the administration of viosterol. This rat showed a definite calcareous deposit in the outer third of the aortic media, which appeared in the hematoxylin and eosin sections to be in the forms of an incrustation on the elastic fibers and a diffuse calcification of several of the muscle cells situated between the elastic fibers. The coronary vessels appeared to be normal.

Series 2 consisted of five animals which received viosterol for from twenty-eight to thirty-eight days. It was administered by mixing it with their food. The aortas of three of these animals showed calcareous deposits. These were found in greatest numbers in the arch and fairly frequently in the upper part of the descending aorta. The animals given an injection of alizarin red showed clearly, in the gross, pinkish areas in the wall of the vessel. On section, a rather patchy distribution of the lesions was found, areas of calcareous deposit being scattered in the inner, middle and outer thirds of the media. When the lesion was extensive, it was usually located in the inner third of the media. In the hematoxylin and eosin preparations, the lesions showed as incrustations along the elastic fibers together with scattered calcifications of the area normally occupied by muscle cells and fibroblasts between the elastic fibers. At the site of a lesion, the elastic fibers were often spread widely apart, sometimes to two and three times the normal distance. In the animals showing calcification of the aorta, the cardiac musculature showed patchy areas of calcification, which were often associated with necrosis of tissue and infiltration by mononuclear and polymorphonuclear leukocytes. The branches of the coronary vessels afforded perhaps the best illustration of the calcareous lesion seen in the animals. In the sections stained with hematoxylin and eosin, these vessels stood out strikingly as edematous, amorphous, granular, blue-colored rings, when cut in cross-section, in which nuclei and cell outlines could be identified only infrequently. The muscle cells of the media appeared to be affected to the greatest extent, and the region about the coronary vessels often showed marked inflammatory cell infiltration associated with calcium deposits. The incinerated sections showed the coronary vessels standing out as bright rings containing greatly increased amounts of mineral matter. The plaques in the aorta were also seen to contain large amounts of mineral matter. The elastic fibers, which showed little mineral matter in normal sections, were also seen to become involved in the process and on occasion appeared to be heavily infiltrated with mineral.

The sections fixed in Flemming's solution and stained with osmic acid showed only a slight amount of fat in the calcareous lesions, nothing of any note. Frozen sections stained with scarlet red showed the same results.

Thus the lesions encountered in this series of animals showed edematous areas in the aortic wall containing large amounts of mineral, probably calcium, which was evident in the degenerating and necrotic muscle tissue, but which was most marked at the periphery of these lesions, where it formed incrustations on the adjacent elastic fibers. In many instances, the latter appeared to be completely calcified as well, demonstrating fractures and other evidences of degeneration and necrosis. The coronary vessels were most markedly calcified, the muscular media being the chief site of involvement. Furthermore, patchy areas of the cardiac musculature were also calcified, and both these areas and the coronary vessels

showed inflammatory cells present about them. The greater calcification in this series over the first series may be ascribed to the method by which the viosterol was administered.

In series 3, each experimental animal received one single dose of activated ergosterol 10,000 X. The first animal was killed forty-eight hours after receiving a single dose; it showed extensive calcifications of the coronary vessels together with patchy areas of calcification in the cardiac muscle and aorta. The remaining six animals of this series died at approximately the end of ninety-six hours; they also showed extensive pathologic lesions. The most striking feature of the picture was found in the coronary vessels, which showed their media to be converted to amorphous-like material, which in the hematoxylin and eosin preparations appeared to contain a large amount of calcium, a finding that was substantiated by the incinerated sections, as they showed greatly increased mineral content. Again there was an inflammatory cell infiltration about the coronary vessels as well as



Fig. 1.—*A*, section of normal rat aorta; hematoxylin and eosin stain;  $\times 250$ . *B*, incinerated section of normal aorta; dark field illumination;  $\times 250$ . The elastic fibers may be seen as clear bands between the muscle cells and fibroblasts.

some patchy inflammatory areas in the cardiac musculature not intimately related histologically to vessels but demonstrating calcification.

Because of the relatively swift production of the lesion, it was thought advisable to duplicate the procedure used in series 3 with a slightly smaller dose and kill animals each twenty-four hours in order to find the preexisting picture corresponding to the characteristic one found in series 3. Consequently, in series 4, ten animals were utilized, and two were examined each twenty-four hours. Photomicrographs of this series are to be seen in figures 2 and 3.

At twenty-four hours, in series 4, the hematoxylin and eosin sections showed no obvious change from the normal (fig. 1). Incinerated sections showed the muscle fibers and coronary vessels to possess approximately the same mineral content as the normal ones. The aorta showed a regular distribution of elastic tissue with no obvious increase in mineral in the muscle cells of the media (fig. 2 *A* and *B*).



At forty-eight hours, lesions were prominent. In the stained sections, the coronary vessels showed marked thickening caused by an edematous amorphous change of the musculature of the vessels, which took a deep blue stain with the hematoxylin. Patches of musculature, usually but not always related to vessels, also showed this change in the cytoplasm of the cells, and these areas as well as the vessels showed an inflammatory cell infiltration about them. Sections from



Fig. 2.—*A*, section of rat aorta twenty-four hours after administration of activated ergosterol; series 4; hematoxylin and eosin stain;  $\times 250$ . No obvious degeneration is evident. *B*, incinerated section from same block as *A*; dark field illumination;  $\times 250$ . The elastic fibers are still seen as clear bands. *C*, section of aorta forty-eight hours after administration of activated ergosterol; series 4; hematoxylin and eosin stain;  $\times 250$ . The characteristic lesion may be noted. *D*, incinerated section from same specimen as *C*; dark field illumination;  $\times 250$ . Note the great increase in mineral content in lesion.

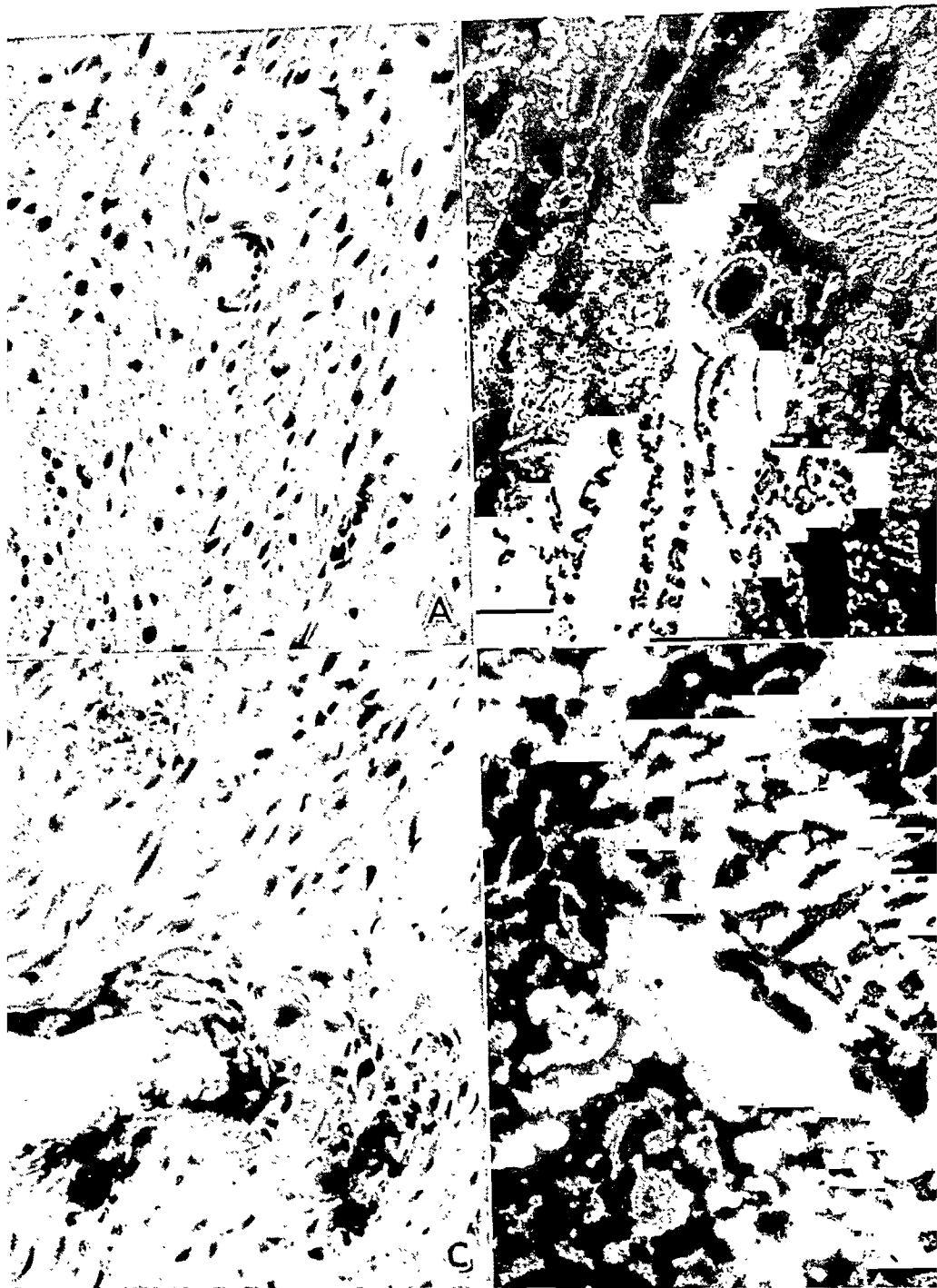


Fig. 3.—*A*, section of cardiac muscle and coronary vessel twenty-four hours after administration of activated ergosterol; hematoxylin and eosin stain;  $\times 250$ . No evidence of calcification or of marked degeneration is present. *B*, incinerated section from same specimen as *A*; dark field illumination;  $\times 250$ . Note the normal distribution of mineral. *C*, section of cardiac muscle and coronary vessel forty-eight hours after administration of activated ergosterol; hematoxylin and eosin stain;  $\times 250$ . The calcification of the vessel and part of the cardiac muscle may be seen together with the inflammatory cell infiltration. *D*, incinerated section of same preparation; dark field illumination;  $\times 250$ . Note the great increase in mineral matter in the wall of the vessel and in certain areas of cardiac muscle.

the arch of the aorta showed edematous areas in the inner third of the media, which were situated between adjacent elastic fibers. These showed areas of deep blue staining, which was deepest along the elastic fibers (figs. 2 *C* and 3 *C*).

The incinerated sections showed a striking series of pictures. The coronary vessels in the heart stood out as thick-walled tubes of mineral matter (fig. 3 *C* and *D*). The change from the picture twenty-four hours earlier was remarkable.

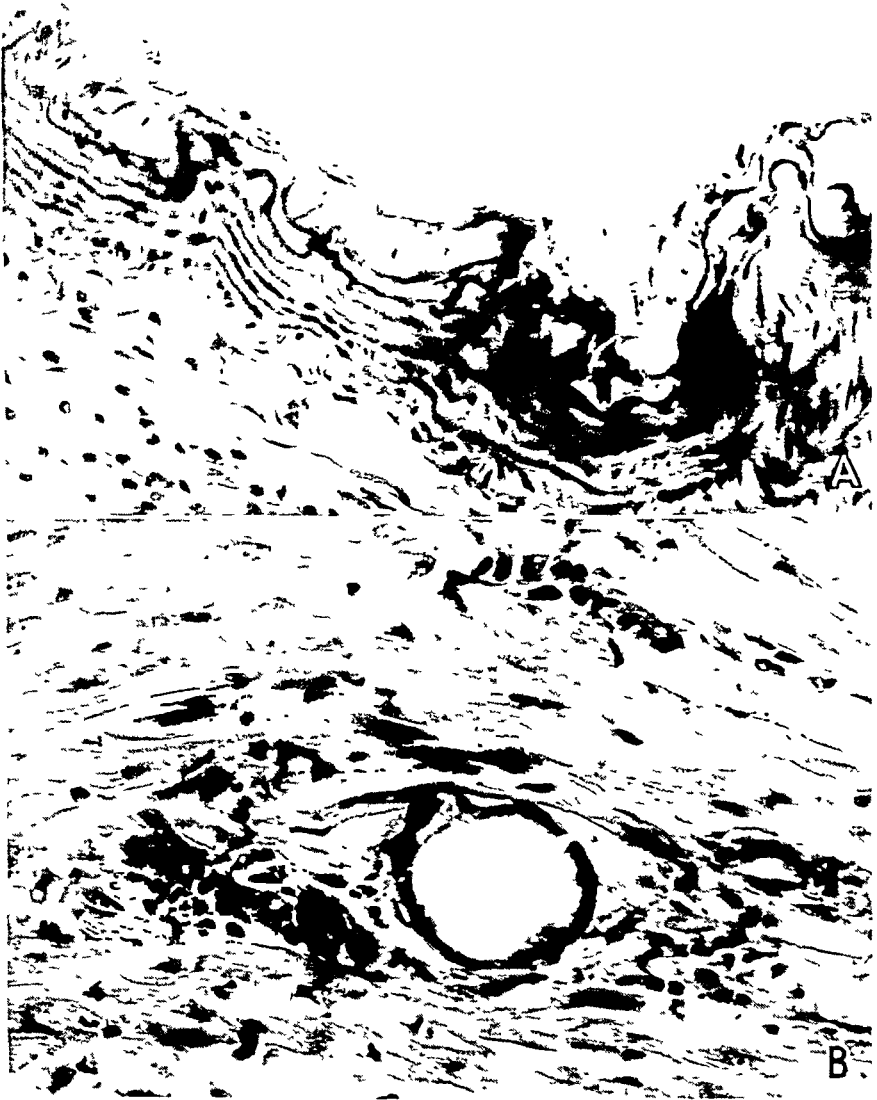


Fig. 4.—*A*, typical lesion produced in aorta by a single enormous dose of activated ergosterol; hematoxylin and eosin stain;  $\times 200$ . *B*, typical picture of calcified coronary vessel, calcified cardiac muscle and inflammatory cell infiltration produced by one single enormous dose of irradiated ergosterol; hematoxylin and eosin stain;  $\times 300$ .

The cardiac musculature also revealed the patches observed in the hematoxylin and eosin sections to be heavily infiltrated with mineral (fig. 3 *C* and *D*). The aorta showed the edematous patches in the inner third of the media to be also heavily infiltrated with mineral matter (fig. 2 *C* and *D*).

At seventy-two hours, much the same type of picture was shown as that observed at forty-eight hours. Each section of the heart showed the same type of inflammatory cell infiltration about the coronary vessels, and the latter as well as the aorta showed relatively huge amounts of mineral deposit.

Before attempting to discuss the significance of the results obtained in this work, the method by which calcium was demonstrated should be commented on. It is apparent that there was, in the sections, a fairly close correlation between the deposits stained by hematoxylin and the deposits shown by the incineration technic to be mineral matter. Furthermore, these same plaques in animals given an injection of alizarin red were colored, so that there seems to be good reason to believe that hematoxylin demonstrates fairly well rather massive deposits of calcareous material when these are formed in the body.

On the other hand, there is good reason to suspect that the incinerated pictures rather minimize the increase of calcium which occurs in the pathologic lesions. The normal incinerated section demonstrates all the mineral ash, only a part of which is calcium, but it is probable that the increase in mineral content at the site of the lesions is almost all calcium, so that the increase in calcium is probably much greater than is apparent.

#### COMMENT

It has been stated previously that an important problem concerns the calcifications of hypervitaminosis D, whether they belong to the group dependent on degenerative changes in the recipient tissues or to the group dependent on the inability of the serum to retain all its calcium even in the vicinity of normal tissues. Calcifications of this second type are not uncommonly seen in certain examples of hyperparathyroidism. They have been produced experimentally with excessive doses of parathyroid hormone by both Leaner<sup>12</sup> and Hueper.<sup>13</sup> Katase<sup>14</sup> described calcifications of this sort which were produced by injecting calcium salts into the body by several different routes. Furthermore, Rabl<sup>15</sup> and later Butler<sup>16</sup> produced calcifications in mice on a high calcium intake by alternating acid and basic diets.

On turning to the experiments performed in this particular work, it is evident that the striking feature was the extreme rapidity with which calcifications of the aorta, coronary vessels and cardiac musculature could be produced. Only second in importance is the fact that twenty-four hours after the administration of the activated ergosterol and only

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12. Leaner, A.: *J. Lab. & Clin. Med.* **14**:921, 1929.

13. Hueper, W.: *Arch. Path.* **3**:14, 1927.

14. Katase: *Beitr. z. path. Anat. u. z. allg. Path.* **57**:516, 1914.

15. Rabl: *Virchows Arch. f. path. Anat.* **245**:542, 1923.

16. Butler, M.: *Proc. New York Path. Soc.* **24**:79, 1924.

twenty-four hours before the appearance of massive calcifications, there were no marked degenerative lesions in the described tissues which would presage such an imminent catastrophe. Furthermore, the massiveness of the calcification together with the previous points commented on would indicate almost beyond question that the calcifications of hypervitaminosis D produced by this experimental procedure belong to the type of calcification that is dependent on the inability of the serum to retain all its calcium, rather than on preliminary degenerative changes in the recipient tissues.

This conclusion seems to be the only one that would be in harmony with other recent observations regarding the toxic action of the vitamin. Bills and Wirick<sup>17</sup> showed that the toxicity of irradiated ergosterol could be intensified by increasing the amounts of calcium in the food. More recently Harris and Innes<sup>2</sup> performed some exceedingly interesting work in this direction and may be quoted as follows:

An increase in the calcium content of a diet intensifies the severity of the hypervitaminosis and gives rise to an increased formation of the calcareous deposits, at a given level of vitamin D excess. With diets virtually devoid of calcium and phosphorus, on the other hand, a hypervitaminosis of a distinctive character can still be produced provided now that the level of vitamin D excess is sufficiently raised; under these conditions calcareous deposits are not in evidence but there is a greatly increased resorption of bone substance.

These findings appear to indicate that the calcifications seen in hypervitaminosis depend, not on the direct action of the vitamin on the tissue, but on its ability to so disturb the calcium metabolism that calcifications are produced. Therefore in contrast to the sequence of events usually observed in the calcifications that occur under a normal calcium metabolism, it appears that in hypervitaminosis D the degenerations observed in the lesions are a result of the disturbance of calcium metabolism, if not of the deposition of calcium.

In considering the manner in which calcifications of normal tissue may be produced by means of alterations of the calcium metabolism, a rather complicated problem is encountered. The calcifications do not appear to depend on simple hypercalcemia. It should be remembered that normally the blood contains more calcium than can be explained by the laws of simple solution. Furthermore one must visualize a mechanism by which the blood is able to build up a high calcium content and yet be unable to retain it in solution. Many of the data regarding the manner in which calcium is carried in the blood have been reviewed and discussed in previous contributions (Ham<sup>18</sup>) as well as the hypothesis which relates to the equilibrium that exists between the

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17. Bills, C. W., and Wirick, A. M.: *J. Biol. Chem.* **86**:117, 1930.

18. Ham, A. W.: *Cartilage and Bone*, in Cowdry: *Special Cytology*, ed. 2. New York, Paul B. Hoeber, Inc., 1932; *Angle Orthodontist*, to be published.

nondiffusible calcium of the blood, the diffusible calcium of the blood and the calcium of the bones. The uncertainties that exist regarding some phases of the calcium metabolism render an understanding of the mechanism of precipitation rather difficult, but there seems to be sufficient evidence to describe at least three possible mechanisms by which the blood could acquire more calcium than it could retain at a later time. These will now be briefly considered.

1. Wells<sup>6</sup> described a mechanism which could account for the precipitation of calcium in the tissues in cases of hypercalcemia by considering that saturated serum of high carbon dioxide content would be forced to release calcium if the reaction of the serum became more alkaline. This mechanism would account for the deposition of calcium in the lungs, if serum saturated with calcium passed through the lungs and lost carbon dioxide, and this explanation has been used to account for the calcifications which appear to occur in cases of hypercalcemia in other areas of acid excretion. It should be remembered, however, that the serum normally contains more calcium than can be accounted for by the laws of simple solution, and as it is very probable that the serum calcium exists in both a nondiffusible and a diffusible form, it is also probable that this explanation instead of applying to the total serum calcium concerns more particularly the diffusible form.

2. A second factor which also comes into play regarding the solubility of the calcium of the blood is well illustrated by findings that have been recorded in certain cases of hyperparathyroidism by Bulger, Dixon, Barr and Schregardus.<sup>19</sup> They pointed out the tendency for a reciprocal rise and fall of the calcium and phosphorus levels, and showed that the blood calcium level could be lowered by the administration of sodium *ortho*-phosphate. If one applies the theory concerning the solubility product of electrolytes in solution, it is apparent that when the ionized calcium of the blood is near the saturation point, the addition of further ions to the serum could easily result in the precipitation of the less soluble calcium salts. These authors suggested this possibility, and the recent work of Hess, Benjamin and Gross,<sup>20</sup> who administered sodium bicarbonate in experimental hypercalcemia, and found that this procedure not only lowered the blood calcium level but caused an increase in the amount of calcium in the tissues, tends to support this hypothesis.

3. Both the preceding theories could account for the precipitation of calcium from serum if the diffusible calcium was at or near the saturation point. Consequently the factors that allow the diffusible calcium to attain the saturation point are of distinct importance. In this

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19. Bulger, H. A.; Dixon, H. H.; Barr, D. P., and Schregardus, O.: J. Clin. Investigation **9**:143, 1930.

20. Hess, A. F.; Benjamin, H. R., and Gross, J.: J. Biol. Chem. **94**:1, 1931.

regard a rather complicated situation is seen to exist, and as yet there is no absolute proof for the following hypothesis, but as there is much evidence that points in its direction it was thought advisable to present it.

It should be remembered that there is not necessarily a relationship between the level of the total serum calcium and the precipitation of calcium from the serum; that is, calcifications do not necessarily appear to result from hypercalcemia. Shelling<sup>21</sup> observed that parathyroid-ectomized rats more readily show calcifications following excessive administration of vitamin D than do normal rats. On the other hand, although the level of the total serum calcium does not appear to bear a distinct relationship to the development of calcifications, there may be a very close relationship between the level of the diffusible calcium and the institution of calcifications. There is good reason to believe that the nondiffusible and the diffusible calcium do not keep their normal relationships in hypercalcemia (Morgulis and Perley<sup>22</sup> and others), as during the attainment of hypercalcemia there appears to be a shift of calcium ions from the diffusible to the nondiffusible and also a shift from the intestine or bones to the diffusible, so that both are raised, but the greater increase is in the nondiffusible calcium. When, however, hypercalcemia is attained, if there is no further administration of parathyroid hormone or Vitamin D, the nondiffusible calcium begins to break down, and this break-down could easily be accompanied by a rather steady release of calcium ions, which could quickly bring the diffusible calcium to the saturation point and beyond this point result in precipitations (Ham<sup>18</sup>). Thus it is entirely possible that calcifications would most readily occur on the down-swing of the serum calcium curve. This hypothesis could explain the calcifications being produced in parathyroidectomized animals more readily than in normal animals, because the fall of the temporarily increased serum calcium would be greater than it would be if the animal had a stable parathyroid mechanism. It also could explain the latent period that occurred before the onset of calcifications following one single dose of irradiated ergosterol in both Laas' experiments and the ones reported in this paper. This hypothesis would also indicate that oscillations of the serum calcium level would be more likely to result in calcifications than steadily maintained simple hypercalcemia. In other words, it seems not unlikely that there is a possibility of the diffusible calcium being kept close to the saturation point, as the serum calcium is falling from a hypercalcemia peak by a continued release of ions from the nondiffusible calcium, and any condition that would allow for saturation of

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21. Shelling, D. H.: *Proc. Soc. Exper. Biol. & Med.* **28**:303, 1930.

22. Morgulis, S., and Perley, A. M.: *J. Biol. Chem.* **88**:169, 1930.

the serum with diffusible calcium would allow calcifications to ensue not only by the previously described mechanisms but also by the process in itself.

The rather characteristic lesions produced in both the arteries and the cardiac musculature (fig. 4), it is thought, are best explained by the rather massive amounts of calcium that are deposited. On the other hand, one must remember that the administration of vitamin D in enormous amounts could first cause a withdrawal of calcium from the bones and possibly other tissues into the blood. There appears to be good reason to suspect that the calcium metabolism of cells could be considerably disturbed not only during the deposition of calcium, but also in the period of withdrawal antecedent to the deposition. In the sections studied in this work, the obvious lesions were always associated with a deposition of calcium, and the early degenerative signs described by many authors were not obvious. On the other hand, it is admitted that the rather severe methods used in this work were conducive to marked lesions, and it is possible that by adjusting dosages and intake of calcium lesions could be produced by the withdrawal of calcium from tissues during the phase of the rise in the blood calcium level. It is, of course, well known that the reaction of the cells of bone in response to a withdrawal of calcium from the matrix by parathyroid hormone or irradiated ergosterol is marked, so that tissue changes in other cells of the body could logically be expected. In this work, however, the well marked lesions were the edematous calcium-containing area between the elastic fibers in the aorta, with degeneration and calcification of both cells and fibers, the calcification of the wall of the coronary vessels, and zones of calcification and inflammatory cell infiltration in the cardiac muscle.

It appears interesting that a picture of myocarditis can be so readily produced by disturbing the calcium metabolism. Of course, these areas of inflammatory cell infiltration are associated with depositions of calcium, but there is a possibility that with less severe doses the inflammatory patches might be produced without such obviously large amounts of calcium. The predominant cells in the exudate are large mononuclears, and there are on occasion polymorphonuclear leukocytes. There is a marked tendency for the lesions to be situated about the coronary vessels.

#### SUMMARY

Enormous single doses of irradiated ergosterol will produce massive calcifications in the aorta, coronary vessels and cardiac musculature of the rat as soon as forty-eight hours after administration. Sections from the tissues twenty-four hours after administration show nothing that would presage such an imminent catastrophe, so that the calcifications



do not appear to depend on degenerative changes in the recipient tissues. On the other hand, the rapidity of formation, together with the massiveness of the calcifications, suggests very strongly that the prime factor in their causation is the inability of the serum to retain all its calcium in solution. It is suggested that precipitation depends on saturation of the serum with diffusible calcium plus other factors. These could be a change in carbon dioxide tension, the addition of other ions which would force a precipitation of calcium salts, or a continued liberation of ions from the nondiffusible calcium after the diffusible calcium had reached the point of saturation. It seems probable that conditions suitable for precipitation could be more easily obtained as the serum calcium level is falling after the attainment of hypercalcemia.

A marked inflammatory cell infiltration developed about the affected coronary vessels and about the calcified areas of cardiac muscle.

The evidence indicates that the toxic action of vitamin D does not depend on a quality separate from that on which its therapeutic action depends, and that the vitamin does not necessarily possess a specific toxic effect on tissues, but that the calcifications of hypervitaminosis D can be explained by its action on the calcium metabolism.

It is also suggested that the calcifications of the variety that depend on the inability of the serum to retain all its calcium in solution depend for their causation to a greater extent on the level of the diffusible calcium than on the level of the total serum calcium.

It should be clearly indicated that the so-called toxic effect of vitamin D demonstrated in these experiments was obtained only with enormous amounts of the vitamin, and that the doses used were infinitely beyond those utilized therapeutically in the administration of cod liver oil or viosterol.

# CONGENITAL CYST OF THE LUNG

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The first description of a case of congenital cyst of the lung has been accredited to Nicolaus Fontanus.<sup>1</sup> Since his time a number of cases have been reported, and upward of fifteen names have been proposed for the condition. While many of these are based on incorrect data, certain features stand out rather prominently. In the cases in which the cysts are large, death often occurs *in utero*, during infancy or at best usually before the age of 25 years. In those in which the cysts are not large the average age attained is 55 years and exceptionally 84 years (Buchman<sup>2</sup>), although death may occur in infancy owing to rupture of a small cyst with resulting pneumothorax (Miller<sup>3</sup>). The condition may involve only one or both lungs, or a whole lobe or a part of one, or it may spring from the parietal pleura entirely unassociated with pulmonary tissue. There may or may not be a demonstrable connection with the bronchial tree (Pappenheimer<sup>4</sup>). There may be an associated malformed artery (Rosenthal<sup>5</sup>), hemihypertrophy of the body (Arnheim<sup>6</sup>) or neoplasia (Heller<sup>7</sup>) and the distended bronchi may or may not be pigmented. The presence or the absence of cartilage in the walls of the bronchi and the character of the epithelial lining have been utilized as points in determining the intra-uterine time at which the error in development occurred. The cystic cavities in Dustin's case were lined by multinucleated giant cells.<sup>7a</sup> Careful study of the reported cases utilized in classifications shows that there is little basis for subdividing them.

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From the Pathological Laboratories of the Jefferson Medical College and Hospital, aided by the Martin Research Fund.

1. Meyer, H.: Virchows Arch. f. path. Anat. **16**:78, 1859. Bartholinus, Thomas, cited by Malpighi, Marcello: Opera omnia seu thesaurus locupletissimus botanico-medico-anatomicus, viginti quatuor tractatus complectens et in duos tomos distributus (complete works), Leyden, P. van der Aa, 1687, vol. 2, p. 349.

2. Buchman, E.: Frankfurt. Ztschr. f. Path. **8**:263, 1911.

3. Miller, R. T.: Arch. Surg. **12**:392, 1926.

4. Pappenheimer, A. M.: Proc. New York Path. Soc. **12**:193, 1912.

5. Rosenthal, S. R.: Arch. Path. **12**:387, 1931.

6. Arnheim, G.: Virchows Arch. f. path. Anat. **154**:300, 1898.

7. Heller, A.: Deutsches Arch. f. klin. Med. **36**:189, 1884-1885.

7a. Dustin, A. P.: Arch. de biol. **42**:229, 1931.

Many have been so poorly studied that only outline descriptions exist, and because of the ambiguity of these the cases have readily lent themselves to such classification. Congenital cysts generally come to autopsy unrecognized or undiagnosed (Koeckert,<sup>8</sup> Müller<sup>9</sup>). The following case is presented because of the association of an interesting physical sign and also because the opportunity of making a detailed morphologic study of the affected area was utilized.

#### REPORT OF CASE

*Clinical History.*—A white woman, aged 54, entered Jefferson Hospital, in the service of Dr. Burgess Gordon, on Nov. 21, 1930. She had always been strong and healthy and had worked hard as a housewife and as a domestic servant. Her best weight had been 150 pounds (68 Kg.), eighteen years before her admission to the hospital. During her entire life she had coughed and expectorated moderately. She was not subject to acute respiratory infection, although epistaxis had been fairly frequent. During the past three years she had been a patient in three hospitals because of gradually developing weakness, loss of weight (42 pounds [19.1 Kg.]), increased cough and several heart attacks characterized by palpitation, precordial pain, dyspnea and syncope.

There was deepening of the supraclavicular and infraclavicular fossae; the ribs and sternum were prominent. The chest was flat, more so on the left, and expansion was limited throughout. There were patchy areas of dulness and hyperresonance, also of increased and decreased fremitus. The breath sounds were roughened, prolonged and in places cavernous. Whispering pectoriloquy was heard over several small areas. A mixture of fine and coarse crackling and musical râles was widespread. A sound identical with a pleural friction rub was heard over the entire chest wherever the stethoscope was placed. Respirations averaged between 28 and 40 per minute. The fingers were clubbed, and there was a slight scoliosis involving the lower dorsal and upper lumbar vertebrae. The heart was rapid, impulse feeble; the sounds were muffled except the second pulmonic beat, which was accentuated. The blood pressure was 110 systolic and 60 diastolic. The pulse beats averaged 110 per minute. Occasionally there was pyrexia.

The urine was normal. No tubercle bacilli were found in the sputum. The hemoglobin content ranged from 75 to 105 per cent; the red blood cells were from 4,200,000 to 5,320,000 per cubic millimeter; the color index was 0.9; the white blood cells averaged about 6,000 per cubic millimeter, with a normal differential count. The Wassermann and Kahn tests were not made.

The roentgen films of the chest were described as showing a general involvement of both lungs, which appeared as though thickly sprinkled by small lumps of raw cotton. The bronchial tree was made out with difficulty. The appearance was considered to be that of generalized pulmonary tuberculosis. In another report, the changes were described as follows: "There is a tuberculous infection involving both lungs with cavities at the right apex. Throughout the rest of the right and in the lower three fourths of the left lung there are numerous exudative foci. From the x-ray point of view the lesion is undoubtedly chronic and long standing, but it is not well healed."

8. Koeckert, H. L.: *Am. J. Dis. Child.* **17**:95, 1919.

9. Müller, H., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1928, vol. 3, pt. 1, p. 557.

The diagnosis of pulmonary tuberculosis was made in all the hospitals. Other diagnoses were chronic bronchitis, bronchial asthma and chronic myocarditis.

Death occurred on Jan. 20, 1931, during an attack of acute cardiac insufficiency complicating pneumonia.

*Postmortem Examination.*—The pleural surfaces were glistening and not adherent (fig. 1). Just beneath and showing through the visceral layer were round, gray, slightly elevated, sharply circumscribed nodules averaging from 1 to 3 mm. in diameter. The intervening depressed tissue (from a fraction of a mil-

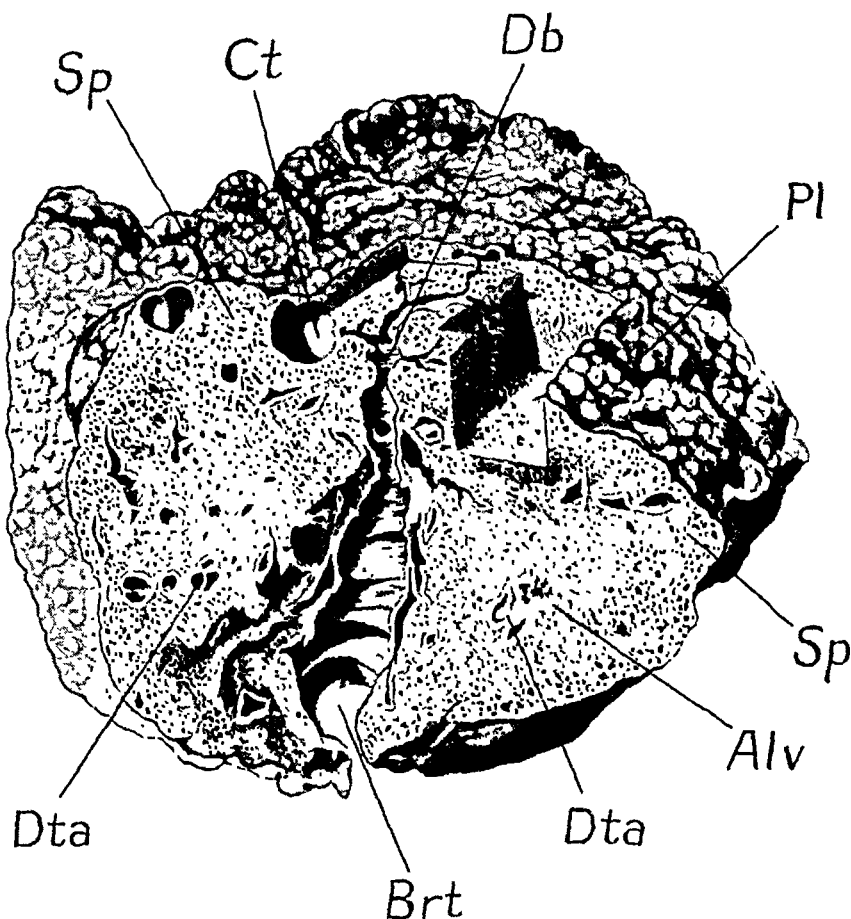


Fig. 1.—Drawing of the lung: *Pl*, pleural surface; *Sp*, subpleural band showing distended bronchi and stroma; *Brt*, dissection of distended bronchus and its branches, showing its subpleural ramification distal to *Db*; *Ct*, large, smooth-lined, cystlike cavity; *Dta*, distended bronchi lying among alveoli, *Alv*.

limeter up to 4 mm. wide) was sclerotic and airless. The nodules were numerous, the picture simulating somewhat that of miliary tuberculosis or that of widespread neoplastic metastases. The lungs cut with increased resistance. On section, these nodules were found to be thick-walled cavities. There were a few subpleural elevated, smooth-lined, thin-walled cysts measuring up to 15 mm. in diameter. Some of these contained as many as five openings, while in others, despite the most careful search, none could be demonstrated. Immediately beneath the pleura, distributed uniformly around the periphery of both lungs, was a layer varying in

thickness from a few millimeters to 3 cm., which was firm and mottled gray and dark red. It consisted of fibrous tissue in which cavities were present. These cavities varied in size from smaller ones barely visible without a magnifying glass to the larger ones already described. On careful dissection it was possible to follow some of these for a considerable distance in various directions. Proximally some communicated with the main bronchial tree. Distally they extended through the fibrous area to the under surface of the pleura, where they ramified parallel or tangential to it. They ended blindly or in a branch too small to be followed. The lumina of these distended bronchi were irregularly widened and narrowed and partially occluded with thick, gray, tenacious mucus. The mucosa everywhere lay

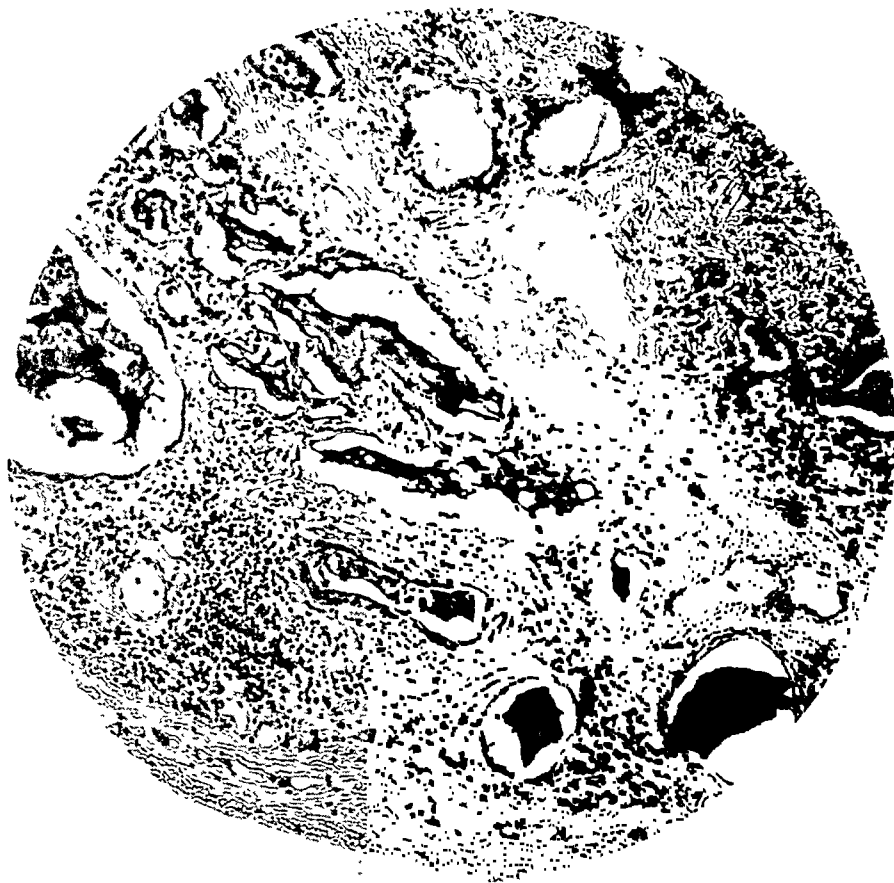


Fig. 2.—Lung. The pleura can be seen at the bottom of the photomicrograph. The character, contents and lining of the distended bronchi and the nodular accumulations of small round cells in the vascular connective tissue are shown;  $\times 100$ .

in fine annular folds. Proximal to this subpleural bandlike area, the lung was consolidated with a recent pneumonic exudate. The bronchi showed cylindric and fusiform dilatations and appeared to be increased in number. The right lung measured 21 by 16 by 6 cm. and weighed 690 Gm. The left lung measured 22 by 18 by 6 cm. and weighed 720 Gm.

Other findings (confirmed by microscopic examination) were: hypertrophy of the right side of the heart (8 mm.), acute myocardial degeneration, hyperplasia of the splenic follicles, parenchymatous degeneration of the kidneys, acute membranous

and ulcerative colitis with melanosis, cholelithiasis, chronic cholecystitis, edema of the lower extremities and passive congestion of the viscera.

Sections were fixed in 10 per cent formaldehyde, Zenkers' fluid and Klotz' solution. They were blocked in celloidin and paraffin and cut at thicknesses varying from 5 to 200 microns. They were stained with hematoxylin and eosin, van Gieson's and Mallory's connective tissue stains, Verhoeff's elastic tissue stain, Mayer's mucematein stain for mucin, Gram's stain for bacteria in tissues, phosphotungstic acid for fibroglia and Levaditi's stain for spirochetes.

*Microscopic Examination.*—The bandlike layer lying immediately beneath the pleura consisted of various-sized, distended bronchi surrounded by an airless vascular connective tissue, containing small round cells (fig. 2). The distended bronchi varied in diameter from several microns to 15 mm., the majority averaging between 0.5 and 1.5 mm. They were round or oval or sometimes much distorted. They showed irregular, redundant serrations and papillary projections. Acute constrictions appeared in those cut longitudinally. Small budlike sacculations were observed, with invagination of the mucosa and multilocular cystlike formation. The lumens of about half of the bronchi were empty. Others contained desquamated epithelium, and still others were plugged with a stringy, hyaline material, which gave the staining reaction for mucin. In the meshes of this could be seen a few neutrophils, eosinophils and a shreddy granular material which appeared like cast-off cilia. The lamina propria was absent in most of the bronchi, while in others it consisted of varying amounts of connective tissue containing collagen and heavy elastic fibrils. The bronchi were lined by squamous, cuboidal, simple and pseudostratified, ciliated and nonciliated columnar epithelial cells. Transitions from one to the other were not uncommon in sections of single bronchi. Only rarely were mitotic figures encountered. Epithelial desquamation was widespread, and frequently the entire lining was separated *en masse*. In some places where desquamation had occurred, bare muscle fibers projected into the lumen. The walls of most of the distended bronchi were free from glands and pigment. They varied considerably in thickness, and were made up of cellular and hyalinized connective tissue, elastic fibers, cartilage and smooth muscle. Elastic tissue was present in the larger ones and appeared as one or more heavy layers just beneath the epithelium. The muscle in many was proportionately increased in amount, although in some it did not completely encircle the wall. Where cartilage plates occurred, they were for the most part in the walls of the larger bronchi. Some were encountered in the smaller ones, and a few appeared to be lying free in the interstitial tissue unassociated with bronchi. The latter occasionally occupied a position immediately underneath the pleura. In general, the plates were round, oval or crescentic. A representative one measured 1 by 10 mm. Many were composed of adult cartilage cells in normal arrangement. Others consisted of palely and deeply stained cells irregularly disposed in various-sized lacunae. At times the periphery became fibrocartilage and merged insensibly with the surrounding stroma. A few were partially calcified.

The stroma of the bandlike subpleural layer was made up of vascular connective tissue containing carbon pigment, smooth muscle and diffuse and focal accumulations of round cells indistinguishable from small lymphocytes. The connective tissue consisted of young and old fibroblasts separated by an abundance of collagen, fibroglial fibrils and elastic tissue. The muscle that was observed in the interstitial tissue was shown by serial sections to connect in many instances with the hypertrophied walls of the distended bronchi. The vascularization of this atelectatic portion was unusually excessive. For the most part, the vessels consisted of thin-walled, endothelium-lined tubes having the appearance of sinusoids rather than

capillaries. The arteries below 2 mm. in diameter showed a uniform subendothelial hyalinization with some atrophy of the media. The internal elastic lamina was unchanged except for occasional slight atrophy. A few of the arteries were occluded by fresh thrombi. There was no inflammatory cell infiltration, new formation of capillaries or thickening of the vasa vasorum in the coats of the vessels. The arterial sclerosis was neither of the hyperplastic (Rosenthal<sup>5</sup>) nor of the syphilitic (Arrillaga<sup>10</sup>) variety.

The pleura was not thickened. Its mesothelial cells were intact and normal. There was no exudate on it. The irregularity of the surface noted grossly resulted from small adjacent clusters of distended bronchi bulging the pleura outward. The depressions were due to the contraction of the intervening connective tissue. If tubules had been substituted for the bronchi, the picture would have corresponded to that of the renal surface in chronic glomerulonephritis.

The lung proximal to this subpleural atelectatic area consisted of normally formed alveoli, which were filled with an acute pneumonic exudate. There was some overgrowth of the stroma, and small distended bronchi similar to those described in the subpleural portion were occasionally encountered. The walls of the larger bronchi were hypertrophied and fibrotic.

A portion of the left lung was reconstructed in the following manner: A block of tissue was cut out so as to include a piece of pleura 12 by 5 mm. and the lung medially for a distance of 34 mm., including bronchi large enough for their connection with the main bronchial tree to be made out grossly. This piece of lung, which had been preserved in Klotz' solution, was dehydrated and infiltrated with parlodin. One hundred serial sections, each 50 microns thick, were cut in such a way that a strip of the pleura and all the structures beneath it for a distance of 34 mm. were present on each slide. The parlodin was removed; sections were stained with hematoxylin and eosin and mounted in balsam. The slides were numbered serially and studied carefully under the microscope. By means of a projectoscope, sections were magnified eighteen times, and the image was thrown on white blotting paper the average thickness of which was 0.9 mm. A tracing of each bronchus was then made and left unstained. The alveoli were drawn in solid blocks and stained red. The interstitial tissue and blood vessels were disregarded, so that in the model they appear as vacant spaces. The alveolar and bronchial tracings were cut out with a sharp knife and mounted serially (Miller,<sup>11</sup> Schaeffer<sup>12</sup>).

The finished model (fig. 3) measures 61.2 by 21.6 by 9 cm. It consists of two portions. The smaller extends proximal from the pleura for an average distance of 11 cm. and is a reproduction of the subpleural distended bronchi and connective tissue (fig. 4). The larger portion, occupying the remainder, averages 50 cm. in length, and reproduces alveoli, bronchi and connective tissue. From a study of these two parts the origin, structure, course and termination of the subpleural distended bronchi and their relation to functioning alveoli can be determined.

According to the model, the majority of the distended bronchi originating within the block of tissue studied were branches of a bronchus in continuity with the main bronchial tree (fig. 4 *Br*). Where branching occurred, the parent bronchus regularly underwent a saccular dilatation (figs. 1 *Db* and 4 *S*). Two groups of distended bronchi had no bronchial or alveolar connection. They occupied a position

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10. Arrillaga, F. C.: *Cardiacos negros*, Buenos Aires, Philadelphia, Wistar Institute Press, 1925.

11. Miller, W. S.: *Anat. Rec.* **48**:191, 1931.

12. Schaeffer, J. P.: *Anat. Rec.* **5**:1, 1911.



Fig. 3.—Photograph of a reconstruction model of distended bronchi and other structures for a distance of 34 mm. from the pleura (model is 61.2 cm. long).

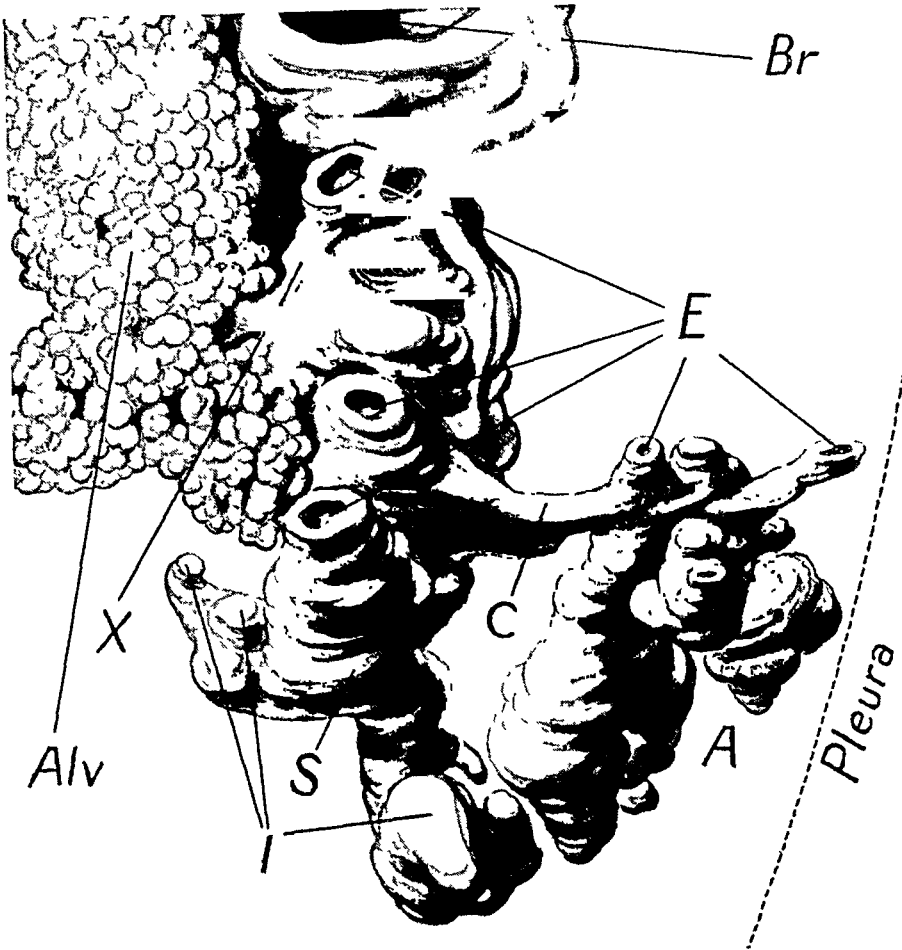


Fig. 4.—Drawing of a reconstructed group of distended bronchi in continuity. They lie immediately beneath the pleura, represented by the dotted line. The group of alveoli (*Alv*) in the upper left corner are fed by the bronchus *X*. *E* indicates bronchi, the lumens of which appear open to represent their termination beyond the block of tissue reconstructed; *I*, bronchi that end within the block of tissue reconstructed; *C*, a small connection between the parent bronchus *Br* (in continuity with the main bronchial tree) and the group of distended bronchi at *A*; *S*, a saccular dilatation.



each point of which was at a sufficient distance from every other bronchus that, had connections been present, they should have been demonstrable.

The progressive gradual tapering was not reproduced with the fidelity that is characteristic of the normal bronchial tree. On the contrary, these bronchi were characterized by coarse dilatations and constrictions as they progressed distally. This distorted them in such fashion that they were frequently narrower at the point of origin than they were some distance away. In addition, there was a fine irregularity which was manifested as a difference in diameter in each successive section of the same bronchus.

For the most part, the subdivisions ran at right angles or parallel to the parent bronchus, either in the same or in the opposite direction. At the periphery, their course tended to parallel the pleura. At variance with this, some described a circular course, some made an acute angle with the parent bronchus and others struck the pleura at a tangent.

A few terminated in functioning alveoli (fig. 4x). The remainder ended blindly, usually in a saccular dilatation, less frequently without any change in the diameter of the lumen and occasionally in a tapered point. Those that terminated in functioning alveoli and those that ended blindly had identical origins.

The bronchi in the proximal portion of the model, while not showing such marked departures from normal, had many of the characteristics of the subpleural distended bronchi. They had small saccular dilatations in their walls, and fine and coarse irregularities in their diameters. They were much larger and more numerous and terminated more abruptly than was normal. Chiefly they supplied functioning alveoli, but occasionally they gave rise to distended bronchi similar in all respects to those found immediately beneath the pleura.

#### COMMENT

The significant symptoms in this case were the cough and the expectoration of life-time duration, due probably to irritation caused by the increased and unexpelled bronchial secretion. Several changes suggest themselves as responsible factors: the desquamation, the flattening of the lining cells with loss of cilia, the alternate constrictions and dilatations in the bronchi, the irregular distribution of the mural musculature, the absence of the tidal wave of air because of the failure of alveoli to form on the terminal bronchi and the interruption of adjacent alveolar contact by the interposition of excessive stroma, thereby abolishing collateral alveolar respiration (Lindskog and Van Allen<sup>13</sup>).

In addition to physical signs indicative of patchy consolidation and of cavitation, a curious sound was heard over the chest, which has been described as that of a pleural friction rub. It was not caused by a roughened pleura, as was proved by necropsy. Occurring as it did with each respiratory movement, it is of diagnostic importance. It may have depended for its production on sounds transmitted through the distended bronchi or on crinkling of the subpleural atelectatic area.

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13. Lindskog, G. E., and Van Allen, C. M.: *Arch. Surg.* **24**:204, 1932.

The roentgenographic changes were interpreted as due to pulmonary tuberculosis. The absence of symptoms comparable with such extensive involvement and the absence of bacilli in the sputum are to be regarded as important diagnostic points.

The associated sclerosis of pulmonary arteries and arterioles, hypertrophy of the right ventricle and polycythemia were interesting. The cardiac hypertrophy was undoubtedly secondary to the resistance offered by the fibrotic lungs and partly to the arterial sclerosis. Whether the sclerosis itself was due to the increased stress (Moschcowitz<sup>14</sup>) or to some other factor is difficult to decide. The polyglobinemia may have been compensatory for the lessened number of alveoli and also for the pulmonary arterial sclerosis (Weber and Bode<sup>15</sup>).

The cells in the subpleural bandlike area suggested an inflammatory reaction, a maldevelopment or a combination of the two. If inflammatory, the process was sufficiently intense that there should not have been the sharp localization over both lungs immediately beneath the pleura. Instead the pleura itself should have been involved, and the process should have converged on the hilus by extension along lymphatic channels. There was no true gummatous formation, but the arrangement of the small round cells into distinct nodules, the vascularity of the lesion and the arterial subintimal hyalinization suggested syphilis (fig. 2). It is more probable that this tissue was embryonic, representing arrested development of alveolar parenchyma, and that the excessive bronchial growth was the result of an attempt to complete this connection. A contributing factor was the normal postnatal bronchial growth (Wilson<sup>16</sup>), also the possible collapse of imperfectly or completely formed alveoli by the prolonged action of the same mechanism responsible for temporary alveolar collapse in asthma (Huber and Koessler<sup>17</sup>).

In the present case there were no signs of congenital syphilis. There was no history of any primary or secondary lesions or of prior administration of specific treatment. There had been no pregnancies. Wassermann and Kahn tests unfortunately were not made. After death neither characteristic gross nor microscopic evidences of the disease were found. No spirochete could be demonstrated in the sections from the lungs nor was the sclerosis of the pulmonary arteries syphilitic in nature. This evidence, while lacking in important essentials, is against

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14. Moschcowitz, Eli: *Am. J. M. Sc.* **174**:388, 1927.

15. Weber, F. P., and Bode, O. B.: *Polycythaemia, Erythrocytosis and Erythraemia*, London, H. K. Lewis & Co., 1929.

16. Wilson, H. G.: *Am. J. Anat.* **41**:97, 1928.

17. Huber, H. L., and Koessler, K. K.: *Arch. Int. Med.* **30**:689, 1922.

syphilitic infection. The presence or absence of syphilis is important because the frequency of visceral maldevelopment in this disease has led to its incrimination in the etiology of congenital cyst of the lung.

The demonstration in the gross specimen and in the reconstruction that certain distended bronchi were devoid of communication with the main bronchial tree confuses their origin. It is probable that at one time a connection existed and that it was later obliterated either by an inflammatory process or by traction of the interstitial connective tissue. Their increase in size would then depend on the pressure of the accumulating secretion which had no outlet.

#### SUMMARY AND CONCLUSIONS

In this case of congenital cyst of the lung, the majority of the distended bronchi communicated with the main bronchial tree. A few had no such connection, and they were therefore true cysts. Evidence is offered that these may have enlarged during postnatal life. The majority of the distended bronchi ended blindly. A few terminated in functioning alveoli. This accounts for the variation in their pigmentation.

As associated factors, hypertrophy of the right side of the heart, sclerosis of the pulmonary artery and polycythemia were found.

The physical sign observed, indistinguishable from a pleural friction rub, is of diagnostic import.

Until more information is available such malformations should be classified under the general term of "congenital cyst of the lung."

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## XI. THE EFFECT OF PHOSPHORUS ON THE NORMAL AND ON THE RESTORED LIVER FOLLOWING PARTIAL HEPATECTOMY IN THE ALBINO RAT

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Anderson<sup>1</sup> recently studied the effect of chloroform on the normal and on the rapidly restored liver, after partial hepatectomy, in white rats, and concluded that the restored liver was more resistant to the effects of chloroform than the normal liver. Lacquet,<sup>2</sup> using carbon tetrachloride, came to similar conclusions. This report concerns a comparable investigation in which I studied the influence of phosphorus on the normal and on the restored liver following partial hepatectomy.

The literature on the effect of phosphorus on the animal organism is probably more complete than that concerning the effect of either chloroform or carbon tetrachloride, and is somewhat older. Of late, phosphorus has not been used so much for experimental purposes, and since the substitution of the relatively inert red for the very active yellow phosphorus in the manufacture of matches, and since the decline in the use of the drug as a therapeutic agent, poisoning is not often encountered. In the following cursory review I shall cite only those more recent contributions to the literature which are pertinent to my study.

According to Luciani,<sup>3</sup> the epithelial cells of the gastro-intestinal canal (like the hepatic cells) have a protective as well as a secretory function. They are able to diminish or to inhibit the effects of toxic substances whether introduced from without or formed within the body. This phenomenon depends not merely on the slow rate at which alkaloids and other toxic substances are absorbed by the intestinal epithelium, but on the fact that after absorption they pass by the radicles of the portal system to the liver, where they are arrested by the hepatic cells, which store up the alkaloids in their cytoplasm, partly restoring them to the intestine with the bile and partly discharging them by the hepatic

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This work was done in the Division of Experimental Surgery and Pathology.

1. Anderson, R. M.: *Arch. Path.* **14**:335, 1932.

2. Lacquet, A. M.: *Proc. Staff Meet., Mayo Clin.* **5**:215, 1930.

3. Luciani, Luigi: *Human Physiology*, New York, The Macmillan Company, 1913, vol. 2, p. 331.

veins for elimination by the kidneys. Schiff<sup>4</sup> found that the fatal doses of narcotic poisons were much lower when introduced hypodermically than when injected directly into the portal vein.

Opie and Alford<sup>5</sup> studied the influence of diet, and found that phosphorus produced fatty degeneration, which was most advanced in the liver. They found that susceptibility to intoxication with chloroform is greatest after a diet of fat, less after one of meat, and least after one of oats and cane sugar. The fatty degeneration produced by phosphorus in the liver was also seen in the kidneys, heart and muscles. Although fat appeared to increase the toxicity of chloroform, meat with equal constancy increased the susceptibility to poisoning by phosphorus. Furthermore, chloroform produced necrosis at the center of each hepatic lobule, whereas phosphorus usually produced widespread fatty degeneration, more intense at the periphery. Phosphorus is less destructive to the hepatic cell, but when given in large doses and especially to animals on a diet of meat, it caused widespread necrosis, more marked at the periphery of the lobule.

Simonds<sup>6</sup> found that in phosphorus poisoning the liver was large and fatty, and that the fatty change began in the peripheral part of the hepatic lobule, but that actual necrosis occurred relatively late. Likewise in eclampsia, fatty degeneration and early necrosis began at the periphery.

The condition in which phosphorus reaches the liver to exert its toxic effect is not known. Plavac<sup>7</sup> (1904) concluded that phosphorus enters into some combination that acts as the poison. Ciaccio and Scaglione<sup>8</sup> studied the effect of chronic phosphorus poisoning on the mitochondria of the choroid plexus, and showed that the mitochondria lost their normal rod shape and became definitely granular.

Simonds found the liver to be the source of much of the intoxication in phosphorus and chloroform poisoning, as well as in acute yellow atrophy. The effects of phosphorus are not manifest until the second or the third day, that is, until the liver is free from glycogen. He suggested that the glycogen protects in phosphorus poisoning possibly by affecting the state of the colloids of the cells; first, by stabilizing the emulsion and preventing its "breaking," with the throwing out of droplets of fat, and second, by reducing the permeability of the cells to the poison.

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4. Schiff, M., quoted by Luciani,<sup>3</sup> p. 331.

5. Opie, E. L., and Alford, L. B.: *J. Exper. Med.* **21**:1, 1915.

6. Simonds, J. P.: *Arch. Int. Med.* **23**:362, 1919.

7. Plavac, V., quoted by Simonds.<sup>6</sup>

8. Ciaccio, C., and Scaglione, S.: *Beitr. z. path. Anat. u. z. allg. Path.* **55**:131, 1912-1913.

Bollman<sup>9</sup> stated that the liver is able to resist better the toxic destruction induced by chloroform, phosphorus, carbon tetrachloride and other drugs, provided it is rich in glycogen, and that more extensive lesions are produced if the liver has suffered previous injury.

#### METHODS OF INVESTIGATION

The white rat was used for this study because of the relative ease with which a large percentage of the liver may be removed and the rapidity with which it is restored after partial removal. The rat withstands partial hepatectomy very well, and no special preoperative or postoperative care is required. Infections are rather uncommon, and throughout the entire series of animals used, peritonitis was rarely encountered at postmortem examination. Dextrose solution was provided the day before and the day of operation in order to secure an adequate glycogen reserve. This enabled the animals to withstand the anesthetic and operation more successfully.

At operation, approximately from 65 to 75 per cent of the liver was removed according to the method described by Higgins and Anderson.<sup>10</sup> An aseptic technic was maintained during all the operations. After operation, all animals were returned to their respective cages, where they remained on the routine diet for one month, in order that ample time might be allowed for restoration of the liver.

In order to determine the toxic dose and the minimal lethal dose of yellow phosphorus for the normal rat, uniform conditions of diet and caging were maintained. Since glycogen is known to offer certain protection, it was necessary that the glycogen content of these livers should be relatively low. In administering phosphorus in the morning prior to feeding, I was assured that the glycogen would be at a minimum. A series of normal rats of approximately the same weight and age were caused to fast for eighteen hours, and were then used in determining the minimal lethal dose of yellow phosphorus for the normal animal. Varying doses of yellow phosphorus in oil (yellow phosphorus in 1 per cent solution of refined cottonseed oil) were injected into the subcutaneous tissues of the abdominal wall. By killing these animals in groups at certain intervals after injection and studying their livers, and by observing mortality among them, it was found that 4 mg. of phosphorus for each 100 Gm. of body weight was the dose of phosphorus that would consistently produce hepatic lesions in normal rats and yet not induce high mortality.

A group of normal rats on the routine diet also were killed in the morning before feeding.

Tissues from all the livers were fixed in two solutions. The tissues selected for fat, connective tissue, hematoxylin-eosin and mitochondrial stains were preserved in 10 per cent neutral formaldehyde, and tissues selected for the del Rio Hortega technic were fixed in an iron and alum solution.

Since phosphorus is excreted from the liver through the bile and reabsorbed from the intestinal tract (Adami<sup>11</sup>), I studied a series of rats in which the common bile duct had been ligated, thus inducing obstruction. One animal was killed seventy-two hours and another one week after the ligation to serve as controls on cytologic changes. The remainder of the series was divided into two groups;

9. Bollman, J. L.: *Proc. Staff Meet., Mayo Clin.* **4**:369, 1929.

10. Higgins, G. M., and Anderson, R. M.: *Arch. Path.* **12**:186, 1931.

11. Adami, J. G.: *The Principles of Pathology*, Philadelphia, Lea & Febiger, 1910, vol. 1, p. 311.

in one group phosphorus was given forty-eight hours after operation, and in the other group, a week after operation. The livers were fixed, stained and studied as described, and the lesions produced were contrasted with those found in the normal animal as well as with those in the animal with the recently restored liver following partial hepatectomy.

#### THE EFFECT OF PHOSPHORUS ON THE NORMAL LIVER

In a series of fifty normal rats that received 4 mg. for each 100 Gm. of body weight and were killed in groups at intervals of one, two, three, four, five, six, twelve, eighteen, twenty-four, forty-eight, seventy-two and ninety-six hours and one, two, three and four weeks, the most outstanding effect in the liver was a

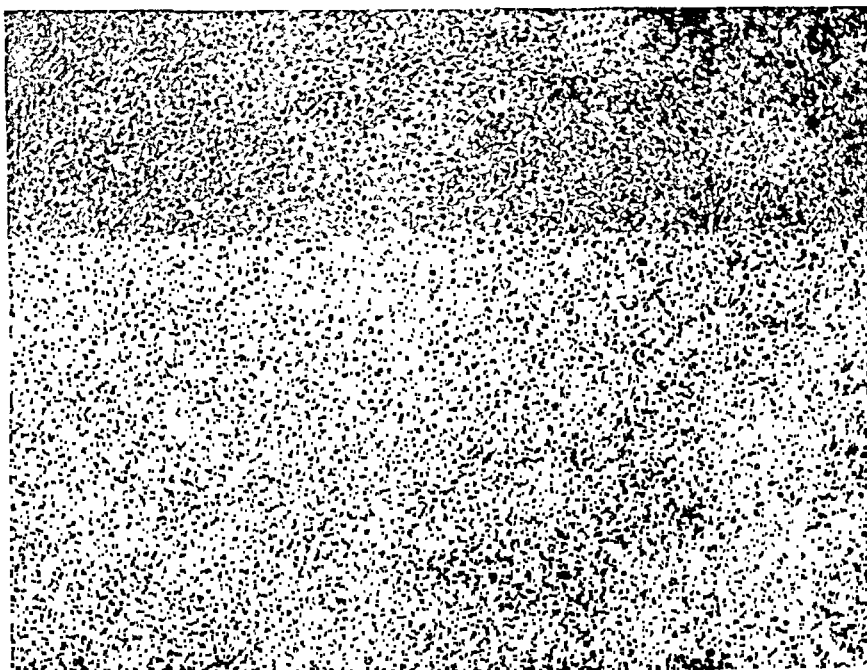


Fig. 1.—The liver of a white rat twenty-four hours after administration of phosphorus (hematoxylin and eosin;  $\times 65$ ).

fatty change. This fatty condition of the liver was observed on opening the abdomen, and at necropsy the amount of fat contained in the liver was estimated and recorded. The fat present was graded on a basis of 1 to 4. A liver in which the fat was graded 1 was considered practically normal, but was slightly yellow on very close inspection, whereas a liver in which the fat was graded 4 was definitely and unmistakably fatty and had the yellow appearance found in the liver of an animal dead of phosphorus poisoning from seventy-two to ninety-six hours after injection.

The animals varied slightly in their reactions to phosphorus in spite of the precautions taken to maintain uniform conditions. The first definite changes in the liver were noted twenty-four hours after the phosphorus was given (fig. 1). The fat, visible microscopically, occurred at the periphery of the lobule and was uniformly distributed in the cell in the form of small droplets.

The normal liver usually contains some fat, which has no characteristic distribution within the lobule, but is scattered here and there in a few cells. Such fat

distribution found either in the normal animals or in the series of animals that received phosphorus was graded 1. In a liver in which the fat was graded 4, every parenchymal cell was heavily laden with fat.

Twenty-four hours after phosphorus had been administered, the fat content of the liver was usually graded 1 or 2. The nucleus was in the center of the cell, or it was crowded to the periphery near its membrane, and there was moderate vacuolization of the cytoplasm of the cells at the periphery of the lobule. There was also slight to moderate cellular infiltration. At forty-eight hours there had been a slight increase in the extent of the injury. The liver contained more fat, and there was more marked cytoplasmic vacuolization (fig. 2). Some cells had been completely destroyed, and the wandering cells about the cell debris were

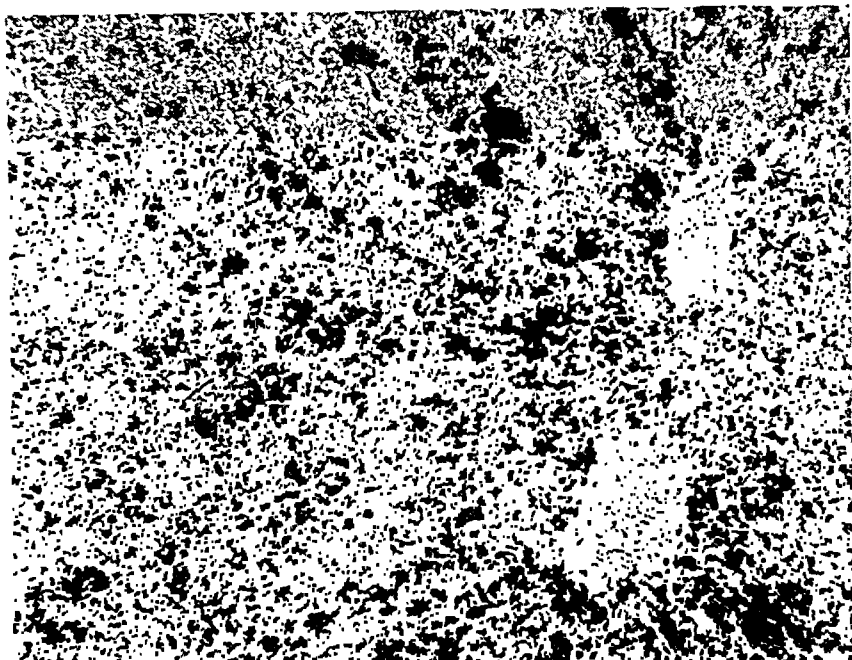


Fig. 2.—The liver of a white rat forty-eight hours after administration of phosphorus (sudan III;  $\times 175$ ).

definitely infiltrating cells. The mitochondria were for the most part granular, decreased in number and equally distributed throughout the cytoplasm.

The most marked changes occurred at seventy-two hours, when the lesion was at its height (fig. 3). Every cell in the hepatic lobule was involved, and the liver for the most part contained fat graded 2 or 3. The fat still appeared most pronounced at the periphery of the lobule, and the hematoxylin and eosin stains disclosed extensive and marked vacuolization of the cytoplasm up to the region of the central vein; in one case every cell was involved. There was cellular infiltration, which was more accentuated in regions in which some of the hepatic cells had been destroyed. Many mitotic figures were seen.

At ninety-six hours, the liver both grossly and microscopically was less fatty than at seventy-two hours. The fat nearest the central veins had disappeared, and the necrotic cells were being replaced by new hepatic cells. Many mitotic figures were still seen. There were considerably fewer mitochondria than normally, and they still appeared as large granules (fig. 4).



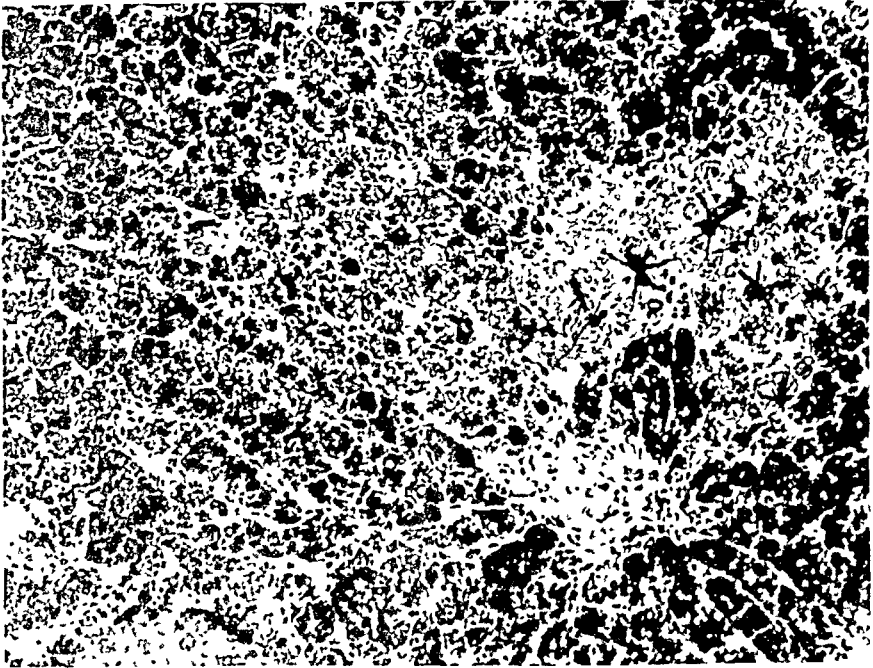


Fig. 3.—The liver of a white rat seventy-two hours after administration of phosphorus (sudan III;  $\times 175$ ).

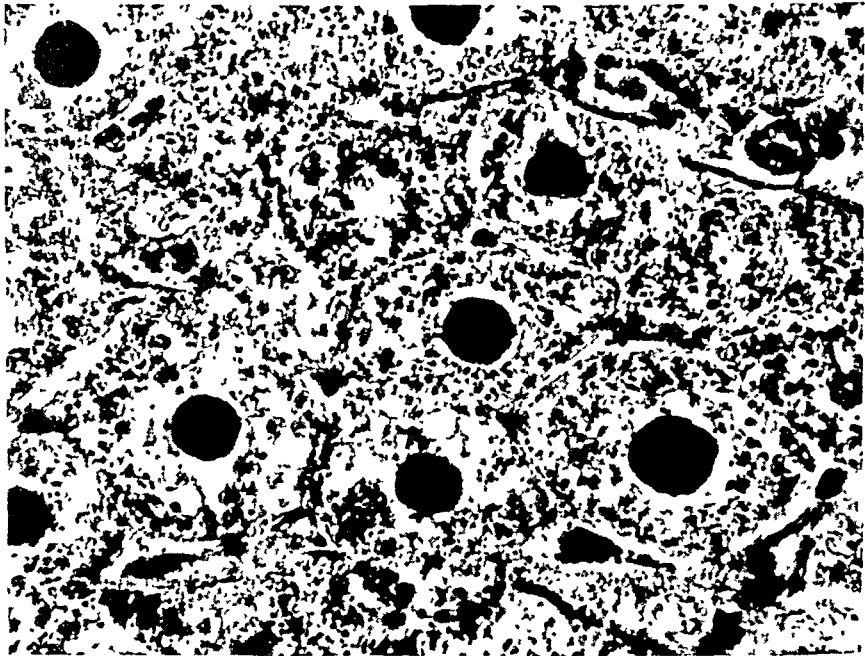


Fig. 4.—Mitochondria in hepatic cells of a white rat ninety-six hours after administration of phosphorus (del Rio Hortega;  $\times 100$ ).

At one week, the liver had essentially returned to a normal condition; most of the fat had been removed, the necrotic cells had been replaced, and the mitochondria again appeared as fine rods and fine granules. At two, three and four weeks, the livers continued normal in every respect.

#### THE EFFECT OF PHOSPHORUS ON THE RECENTLY RESTORED LIVER FOLLOWING PARTIAL HEPATECTOMY

Four milligrams of phosphorus for each 100 Gm. of body weight was given to a series of fifty rats following restoration of the liver after partial removal, and the animals were killed in groups at one, two, three, four, five, six, twelve, eighteen, forty-eight, seventy-two and ninety-six hours and one, two, three and four weeks

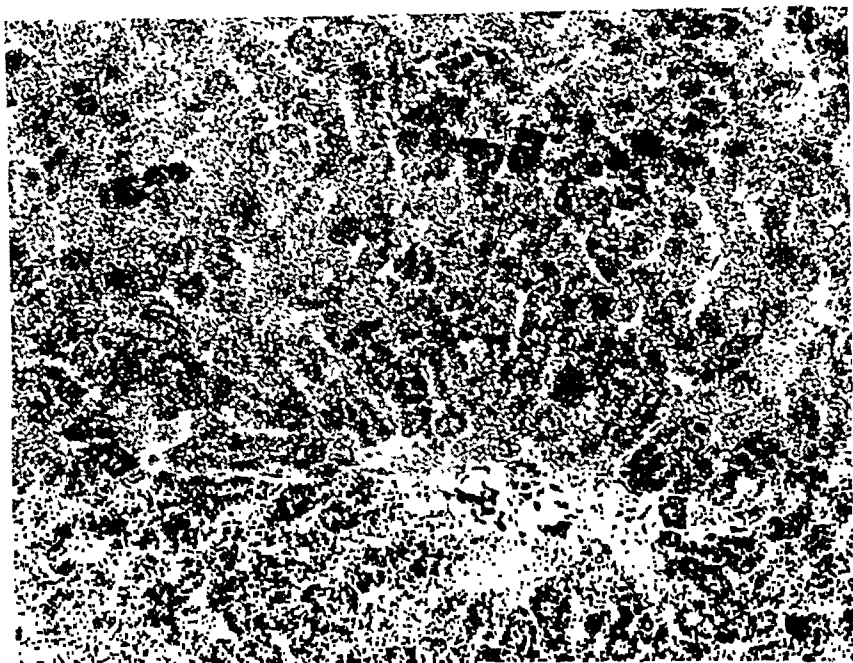


Fig. 5.—The liver of a white rat four weeks after partial hepatectomy and eighteen hours after administration of phosphorus (sudan III;  $\times 175$ ).

after injection. Their livers were studied grossly and microscopically, and the extent of injury was estimated in a manner comparable to that employed for normal animals.

Prior to twelve hours after injection, the animals, with one exception, did not reveal gross or microscopic evidence of a greater deposition of fat than that in the control group. At twelve hours, the fat in the livers of three of the four animals killed was graded 2; the liver of the fourth animal appeared to be normal at eighteen hours (fig. 5). There were slight congestion, some vacuolization and infiltration; the changes were most noticeable at the periphery, and the fat content was graded 2 or 3. The mitochondria were small rods and small granules, but they were apparently somewhat larger than those seen in the normal animal.

At twenty-four hours there was only a slight change from the conditions seen at eighteen hours (fig. 5), and at forty-eight hours conditions were the same, except that the lesions were more extensive and vacuolization of the cytoplasm was more marked. Mitotic figures were already numerous.

At seventy-two hours, the lesions were most marked. The fat in all the livers was graded 3 or 4, and there was extensive vacuolization of the cells, which involved those adjacent to the central vein as well as those at the periphery. There was moderate infiltration by wandering cells, and mitosis was common.

At one week, the fat content of the livers of three of the five animals killed was normal, and cytoplasmic vacuolization was slight. Mitotic figures were numerous, and the mitochondria had returned to normal. The fat in the livers of the other two animals was graded 2.

#### THE EFFECT OF PHOSPHORUS ON THE LIVER FOLLOWING LIGATION OF THE COMMON BILE DUCT

Fifteen normal rats were subjected to obstruction of the common bile duct. The effect of phosphorus on the livers of these animals was studied at intervals ranging from six hours to one week after injection. One group of seven animals received injections of phosphorus forty-eight hours after the common bile duct had been obstructed, and another group of six animals received injections at the end of one week after the operation. One animal was killed seventy-two hours and another one week after operation.

At necropsy, the animals were markedly jaundiced. The peritoneum, liver, kidneys and muscles were distinctly yellow. One week after operation, the extrahepatic ducts and the common bile duct proximal to the ligature were enormously distended, so that the hepatic ducts had a transverse measurement about equal to that of a lobe of the liver. Forty-eight hours after operation, the bile in the ducts was yellowish, but that found at the end of a week was clear and watery. The livers of these animals were so discolored with bile pigments that it was impossible to estimate the amount of fat grossly. Microscopically, however, it was easy to distinguish the fat and to estimate the amount present.

The most striking feature noted in the stained sections of these livers was the marked increase in the number of the bile passages (fig. 6). The picture presented was entirely different from anything observed in the other groups studied. At each portal space there were, instead of the usual one or two, or possibly three, bile passages, a great many new ducts. These ducts were so numerous that in some lobules they extended well beyond the midzone. This description holds for the controls examined at forty-eight hours as well as for those examined at one week; the only difference was one of degree; that is, at one week after operation the extrahepatic ducts were larger and more distended and the contents clearer, and the increase in the number of periportal bile passages was more marked. The new bile passages were supported by very little connective tissue, and mitotic figures in the biliary epithelium were common. The epithelial cells and their nuclei were larger than those in normal livers. The cells of the parenchyma were normal, and there was no evidence of injury or of repair. The fat at seventy-two hours and at one week was graded 1. The droplets were of medium and large size and were scattered about the central vein.

The lesion produced by phosphorus in the livers of these animals in which the common bile duct had been ligated was identical in type and situation with that produced in the normal and in the recently regenerated livers, but there was a distinct difference as to the time of its appearance and the period of its height.

In the group in which injections were given forty-eight hours after ligation of the common bile duct, the lesion was present as early as six hours after the injections. The liver was jaundiced, and the fat was graded 2, with medium and large droplets of fat filling all the cells in the periportal zone. There was slight cellular

infiltration, and the mitochondria were of the large granular type. At twenty-four hours, the fat was graded 3, and the change was far more marked than at six hours. At forty-eight hours, the lesion was less, and the fat was graded 1.

At seventy-two hours, the lesion was similar to that at twenty-four hours, except that the cytoplasmic vacuolization was less pronounced. Mitotic figures were occasionally seen among the hepatic cells. At ninety-six hours and at one week after injection, the livers were again normal as far as could be determined, except that the mitochondria remained as large granules. In fact, in all animals in which the common bile duct had been ligated, the mitochondria were never normal before or after the administration of phosphorus. Likewise in the animals that were given injections one week after the ligation of the common bile duct, the lesion was well marked at six hours, and the sequence was more or less identical with that when phosphorus was given forty-eight hours after operation.

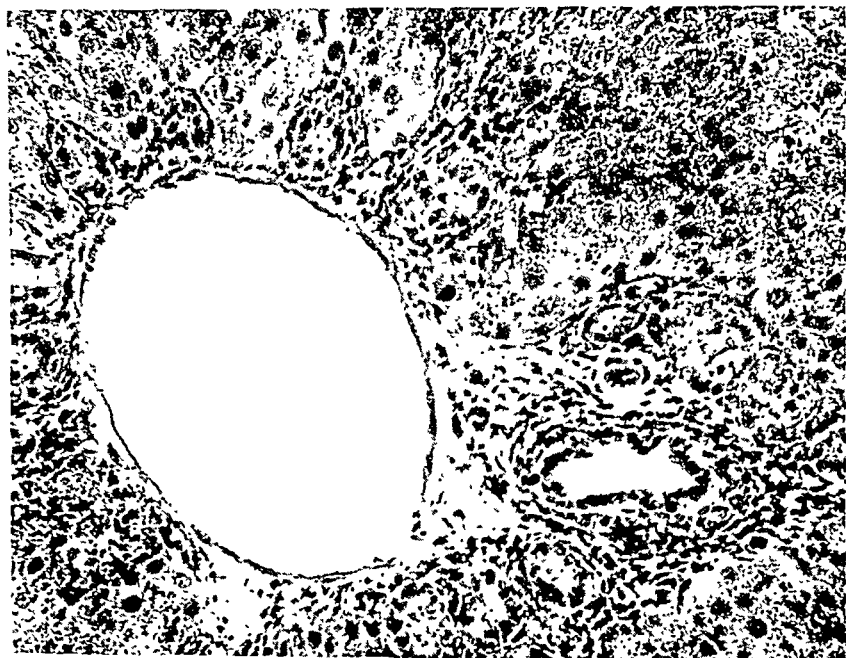


Fig 6.—The liver of a white rat one week after ligation of the common bile duct and six hours after administration of phosphorus ( $\times 175$ ).

#### COMMENT

The amount of yellow phosphorus that I found would consistently produce lesions in the liver of the albino rat was much larger than the amount employed by other workers. The difference in response to a given poison is difficult to explain, although it is known that animals of the same experimental group vary considerably in their response to a given dose of phosphorus.

In the group of normal animals that received the injection, 4 mg. of phosphorus for each 100 Gm. of body weight, the lesion appeared between eighteen and twenty-four hours later at the periphery of the

lobule and gradually extended toward the center, attaining the height of injury at about seventy-two hours.

Necrosis and cellular infiltration were not as prominent a part of the lesion as in carbon tetrachloride poisoning, for example. The lesion consisted mainly of fatty change, which has been designated as fatty degeneration. There is much controversy as to whether this change is really degenerative or the result of infiltration. For the purpose of this study, however, the origin of this fat is irrelevant, since I was primarily interested in knowing whether the recently restored liver following partial hepatectomy differed essentially from the normal liver in response to phosphorus.

Seventy-two hours after injection, when the maximal injury had been reached, the process of repair had begun. The fat disappeared first from the cells at the center of the lobules, and the process of recovery proceeded toward the periphery. A week after injection, the liver had returned to normal. The hepatic cells were again normal in appearance and in fat content, and the mitochondria were fine rods and granules. There was no increase in connective tissue, and the normal structure of the liver was regained, so that there was no distortion or scar formation from any overgrowth of supporting tissue.

Although the fatty change characteristic of phosphorus poisoning appeared in the livers of normal animals twenty-four hours after the subcutaneous injection of phosphorus, it appeared within twelve hours after such an injection in rats with recently restored livers following partial removal. From this time on there was progressive extension of the lesion from the portal spaces toward the central veins, just as in normal animals that had received phosphorus. The most marked lesion, however, was observed at the same interval after injection of phosphorus as in the normal animal, namely, seventy-two hours; but the injury was more slowly repaired in the recently restored organ. At ninety-six hours there was scarcely any demonstrable change in the extent of the lesion, and even at one week after injection there were definite fatty changes in the livers of two of the five animals. At the end of four weeks, severe and extensive fatty change appeared in one animal, which was interpreted as the result of phosphorus poisoning.

After ligation of the common bile duct there was definite injury to the hepatic cells, as indicated by the effects on the mitochondria. The mitochondria, instead of appearing as fine rods and fine granules, were larger and fewer, and were concentrated about the nucleus of the hepatic cell.

After an injection of phosphorus in amounts corresponding to those used in normal animals and in those with recently restored livers, there was a marked difference in the response in the liver in which the common

bile duct had been obstructed. The action of the phosphorus was the same and in the same situation, but its extent, time of appearance and degree of healing varied widely from that observed in the normal and in the recently restored livers. The lesion appeared within six hours after injection, but it was never marked, and it had entirely cleared up by the end of ninety-six hours. The mitochondria, however, never returned to normal, which indicated that there was probably some injury to the cellular constituents of the liver after obstruction to the common bile duct and the resulting jaundice.

Thus the livers in which the common bile duct had been obstructed by ligation apparently were less susceptible to the untoward action of phosphorus. Although the lesion of phosphorus poisoning appeared sooner after injection in this series than in the normal or in the regenerated series, it never was as marked and did not persist as long. This is in keeping with the teaching of Adami, namely, that phosphorus is eliminated in the bile into the intestines, from which it may be reabsorbed, thus producing a vicious circle and prolonging the effect.

From my observations, it appears that the lesion produced by phosphorus in the recently restored liver of the albino rat develops more rapidly, is more extensive and is slower in repair than that induced in the normal liver. Thus, as far as the detoxification of phosphorus is concerned, it would seem that the recently restored liver is far less effective physiologically than the normal liver.

This conclusion is in contrast with the conclusions of Anderson, who used chloroform as the hepatolytic substance. He found that the recently restored liver was less susceptible to chloroform poisoning than the normal liver. In other words, he found that a given dose of chloroform would produce greater injury in the normal liver than in the recently restored liver. Likewise, Lacquet, while working with carbon tetrachloride, found that the recently restored liver was more resistant to chemical injury, in that the lesion produced was far less extensive and recovery ensued far more rapidly than in the normal liver. He found this to be true, however, only when the lesion was induced from two to four weeks after partial hepatectomy, when, as he pointed out, the hepatic parenchyma was exceedingly active and perhaps more embryonic in its qualities. The lesion induced two months after partial removal of the liver was more or less identical with that which Lacquet encountered in a normal liver after the administration of carbon tetrachloride.

I am unable to say whether the fat that appears in the liver after the administration of phosphorus is the result of a destruction of mitochondria, as has been stated by Cowdry (1926), or whether it arises from some other source. Certainly there are changes that take place in

the mitochondria at the time of or just before the fat becomes visible in the cells, but whether the fat originates in or from the mitochondrial substance, I am unable to determine. As the fat disappears the mitochondria return to their normal size and form.

#### CONCLUSIONS

Yellow phosphorus produces a characteristic lesion in the recently restored liver of the albino rat. This lesion cannot be distinguished grossly or microscopically from the lesion produced in the normal liver. It consists of a fatty change, which is always most marked at the periphery of the lobule.

The lesion produced in the recently restored liver by phosphorus makes its appearance sooner, becomes more extensive and is slower in its repair than that produced in the normal liver.

After the common hepatic duct has been obstructed surgically, the untoward or toxic effect of phosphorus on the liver is observed sooner, but the lesion produced is not so extensive as that produced by a similar dose of the drug in the normal or in the recently restored liver. Furthermore, the fatty change disappears in a much shorter time from the liver of an animal in which the common bile duct was ligated prior to the administration of phosphorus. There seems to be little difference whether the toxic drug is administered forty-eight hours or one week after the duct has been obstructed.

# THE PARATHYROID HORMONE

## ITS REGULATORY ACTION ON THE PARATHYROID GLANDS AND TOXIC EFFECT ON THE TISSUES OF THE RAT

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W. R. TWEEDY, PH.D.

AND

H. C. BREUHAUS

CHICAGO

The effects of excessive doses of parathyroid hormone have been described by several investigators, and lesions of the skeletal system, produced by administration of the hormone, have been subjected to intensive study. It was not our plan to repeat these experiments, but rather to determine the acute effects of the hormone on the cells of the parathyroid gland. While the investigation was under way, one of us (W. R. Tweedy<sup>1</sup>) developed methods by which the hormone could be inactivated and the inert product partially reactivated. This suggested observations on the toxicity of the hormone not heretofore possible. Furthermore, it was found that a detailed study of hormonal lesions of the soft tissues of the rat had not been reported.

The first part of this paper deals with the action of the parathyroid hormone on the cells of the gland producing it, and the second part, with the pathogenesis of the lesions produced by the parathyroid hormone.

### ACTION OF EXCESS PARATHYROID HORMONE ON THE CELLS THAT SECRETE IT

That compensatory hyperplasias, hypertrophies and regenerations are responses to physiologic needs is easy to demonstrate, although the mechanism by which these cellular changes are brought about is not clearly understood. Increased blood supply and increased functional demand are accepted as formative stimuli. Other and more specific factors in the regulatory mechanism must be recognized. Loeb<sup>2</sup> and his associates found that the feeding of thyroid inhibited regeneration

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The preparation of a portion of the hormone used in these studies was aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Tweedy, W. R., and Torigoe, M.: J. Biol. Chem. **48**:97, 1932.

2. Loeb, L.: J. M. Research **41**:481, 1920.



of the thyroid remnant after partial thyroidectomy. One of us (F. A. McJunkin<sup>3</sup>) demonstrated the inhibitory effect of insulin on the proliferation of the islet cells of the pancreas. We were therefore interested in making observations on the effect of parathyroid hormone on the parathyroid glandules.

*Methods.*—Histologic: The animals were killed at approximately eighteen hours after the last injection, and the tissues were immediately fixed. We soon found that formaldehyde was unsatisfactory for the fixation of the parathyroid glands of the rat, and that the best results could be obtained by fixing the two glandules, with the minimum amount of thyroid tissue about them, for two hours in a mixture consisting of 8 parts of 2.5 per cent potassium bichromate and 2 parts of 40 per cent aqueous solution of formaldehyde, after which they were placed in a 2.5 per cent potassium bichromate solution for two days. The entire glands, embedded in paraffin, were then cut into serial sections, 8 microns in thickness. With a mechanical stage, mitoses were enumerated, in most instances, in both parathyroid glands. Until experience was acquired, there was difficulty in the identification of certain of the karyokinetic figures. Animals kept on the same rations and in the same environment as the treated ones were used as controls.

Chemical: Before the animals were killed, blood was drawn by cardiac puncture, and the calcium analysis made, in most instances, on a 1 cc. sample of serum. The Kramer-Tisdall<sup>4</sup> method, as modified by Tweedy and Koch,<sup>5</sup> was employed.

An average blood serum value of 10.8 mg. per hundred cubic centimeters was found for twelve normal animals kept on the same rations as the treated animals. Some of the twelve appear as normal controls in the tables, and the others were selected at random. In previous work, one of us (W. R. Tweedy<sup>6</sup>) had found that the normal value for different rats may lie between 9.25 and 12.5 mg. per hundred cubic centimeters. We accordingly interpreted any value greater than 12.5 as above normal.

The hormone preparation designated "L" in the tables is para-thor-mone. The preparation not so designated was prepared by the method of one of us (W. R. Tweedy<sup>7</sup>) and standardized by Collip's procedure.<sup>8</sup> The acid alcohol inactivated hormone preparation is described by one of us<sup>1</sup> in a separate publication.

Information to show that a deficient supply of hormone in the tissues favors proliferation of parathyroid cells was obtained by Halsted,<sup>9</sup> who successfully transplanted parathyroid gland only in those animals in which one half of the parathyroid tissue has been previously removed. One of us (W. R. Tweedy<sup>10</sup>) obtained "takes" only when the blood calcium was below 8 mg. per hundred cubic centimeters.

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3. McJunkin, F. A., and Roberts, B. D.: *Proc. Soc. Exper. Biol. & Med.* **29**: 893, 1932.

4. Kramer, B., and Tisdall, F. F.: *J. Biol. Chem.* **47**:475, 1921.

5. Tweedy, W. R., and Koch, F. C.: *J. Lab. & Clin. Med.* **14**:747, 1929.

6. Tweedy, W. R., and Chandler, S. B.: *Am. J. Physiol.* **88**:754, 1929.

7. Tweedy, W. R.: *J. Biol. Chem.* **88**:649, 1930.

8. Collip, J. B., and Clark, E. P.: *J. Biol. Chem.* **66**:133, 1925.

9. Halsted, W. S.: *J. Exper. Med.* **11**:175, 1909.

10. Tweedy, W. R., and Chandler, S. B.: Unpublished data.

Excess hormone, on the other hand, was found by Jaffe and Bodansky<sup>11</sup> to cause atrophy and distortion of the parathyroid cells. In our animals we could not clearly establish either of these two histologic changes. We used the rat, not the dog, and our experiments were of shorter duration.

*Effect on Mitotic Activity.*—The average number of mitoses per animal in eight control rats was 37.7 (table 1). The action of the parathyroid hormone was decisive. The maximum rate in the animal that received the highest dosage of the hormone (table 2) was less than

TABLE 1.—*Mitoses in Normal Control Rats*

Rat	Weight, Gm.	Glands Counted	Mitoses per Animal	Blood Serum Calcium, Mg. per 100 Cc.
1.....	130	2	23	....
2.....	135	1	27	....
3.....	142	2	13	11.6
4.....	215	2	53	....
5.....	132	2	51	10.5
6.....	100	2	23	....
7.....	125	2	50	....
8.....	153	2	61	....

TABLE 2.—*Mitoses in Hormone-Treated Rats*

Rat	Weight, Gm.	Duration of Experiment, Days	Dosage, Units and Times Injected	Glands Counted	Mitoses per Animal	Blood Serum Calcium, Mg. per 100 Cc.
1	118	3	50 (3×)	2	2	17.1
2	130	7	50 (4×)	1	4	....
3	145	15	10 (12×)	2	3	11.4
4	130	15	10 (12×)	2	1	10.8
5	122	10	50 (3×)L	2	3	12.1
6	110	3	50 (3×)L	2	0	15.2
7	120	3	50 (3×)L	1	6	14.4
8	125	3	50 (3×)L	2	3	15.5
9	130	3	25 (3×)L	2	3	13.7
10	143	3	25 (3×)L	2	4	11.2
11	135	3	25 (3×)L	2	3	12.4
12*	120	3	50 (3×)†	2	5	10.8
13*	105	3	50 (3×)†	2	0	10.7
14*	120	3	50 (3×)†	2	0	10.6
15*	135	3	50 (3×)†	2	5	10.9
16*	160	3	50 (3×)†	1	1	10.8
17*	120	3	50 (3×)†	2	5	10.6

\* Animals 12, 13, 14, 15, 16 and 17 are designated, respectively, as 1, 2, 6, 7, 8 and 9 in table 4.

L = para-thor-mone.

† Inactivated hormone.

half that encountered in any control. In one treated animal no mitoses could be identified, and in another only a single mitosis was present. In the entire series of seventeen animals, the hormone reduced the number of mitoses to an average of 2.8 per animal. The low figures were not confined to the animals that received excessively large doses of hormone administered in a way calculated to give a maximum increase of blood calcium. In two rats (3 and 4, table 2<sup>12</sup>) that received

11. Jaffe, H. L., and Bodansky, A.: J. Exper. Med. **32**:669, 1930.

12. Animals 3, 4, 5, 6, 7, 8, 9, 10 and 11 (table 2) appear, respectively, as 9, 10, 12, 13, 14, 15, 16, 17 and 18 in table 3.

a dozen doses of 10 units each, an amount insufficient to elevate the blood calcium, the gland was affected to as great an extent as in two animals (7 and 8, table 2) in which hypercalcemia was produced. In another rat (6, table 2) with high calcium, no mitosis was found. In animals 3 and 4 (table 2) an undemonstrated hypercalcemia may have been present at one time or another during the treatment. Doses of 25 units, administered on three successive days, effectively inhibited mitotic division, yet the calcium was not much increased (9, 10 and 11, table 2).

TABLE 3.—*Toxic Necroses*

Rat	Weight, Gm.	Dosage, Units and Times Injected	Duration of Experiment, Days	Blood Serum Calcium, Mg. per 100 Cc.	Necrosis of Kidney	Necrosis of Heart	Calcification of Lesions
1	104	15 (4×)	6	13.0	+	—*	+
2	118	22 (4×)	5	14.8	+	—	+
3	85	17 and 34 on alternate days	18	15.4	++	++	++
4	107	75 (1×)	1	13.6	+	—	—
5	91	50 (1×)	1	13.0	—	—	—
6	118	100 (1×)	1	12.6	+	—	—
7	86	100 (1×)	1	9.2	—	—	—
8	130	50 (4×)	7	17.0	++	++	++
9	145	10 (12×)	15	11.4	—	+	—
10	130	10 (12×)	15	10.8	—	+	—
11	142	10 (12×)	22	11.2	—	+	—
12	122	50 (3×)	10	12.1	++	+	++
13	110	50 (3×)L	3	15.1	++	+	++
14	120	50 (3×)L	3	14.4	++	+	++
15	125	50 (3×)L	3	15.5	++	—	+
16	130	25 (3×)L	3	13.6	—	+	—
17	143	25 (3×)L	3	11.2	—	—	—
18	135	25 (3×)L	3	12.4	—	—	—
19†	125	150 (1×)L	2	....	++	+	—
20†	125	150 (1×)L	2	14.8	++	+	+
21	88	18 and 36 on alternate days	16	13.7	++	—*	++

\* Sections were taken across the apex where necrosis was less seen.

† See the text.

*Regulatory Effect on Secretory Activity.*—In young white rats, an amount of insulin insufficient to interfere with normal growth and development effectively inhibits mitotic proliferation of the pancreatic islet cells. From an examination of tables 1, 2 and 3 it appears that there is an inhibition of mitosis in the parathyroid gland by an amount of hormone that is insufficient to produce destructive lesions in the parenchymatous organs (16, 17 and 18, table 3). It is also seen in table 2 that an arrest of mitotic proliferation in the parathyroid gland is present in the absence of demonstrated hypercalcemia. After a consideration of all the data, we are inclined to view the inhibition of mitosis as the most delicate test for excessive parathyroid activity in the rat, the production of lesions in parenchymatous organs as next in this respect, and the elevation of blood calcium as the least sensitive of the three indicators.

Our results establish the efficacy of the hormone in inhibiting the normal proliferation of the parathyroid gland. That the proliferation of the various tissues is not easily inhibited by a great variety of adverse conditions is attested to by the continuous growth of the organism. We venture to advance the opinion that this hormone, as well as other hormones, by automatically acting as a regulator or governor of functional activity, exercises an effect on the cell that secretes it more delicate than the arrest of mitosis. Any excess of hormone, above the functional need, may serve to depress hormonal output, while a deficiency may stimulate output by releasing the normal activity of the cell.

The action of the inactivated hormone is of much interest. Of the three hormonal effects, parathyroid proliferation, destructive lesions and hypercalcemia, the first was the only one observed. It was decisive (table 2), although—judging from the inability to produce hypercalcemia in the dog—the inactivation was complete.

#### TOXICITY OF EXCESS PARATHYROID HORMONE

Collip<sup>13</sup> observed the lethal effect of repeated injections of the parathyroid hormone on the dog. The chief lesion noted was in the stomach, which was described as hemorrhagic and congested. An identical lesion of the stomach was produced by intravenous injection of a mixture of calcium chloride and acid sodium phosphate.<sup>14</sup> Hueper<sup>15</sup> made a histologic study of the various organs of dogs subjected to hormonal overdosage. He stated: "The effect of the hormone on the action of the heart, and the circulatory system, is the cause of the hemorrhages, thromboses, and secondary necroses in several organs." In a consideration of the lesions in the rat, the resistance of this animal to the hormonal effect should be kept in mind. Collip said: "It has been our experience that the normal rabbit and rat are immune to repeated injections of the hormone, and indeed showed very little change in the blood serum calcium values under such treatment." Tweedy and Chandler<sup>6</sup> were the first to offer evidence of hypercalcemia in the rat.

We first observed extensive lesions in rat 3 (table 3), which was treated on successive days with large doses of the hormone. Some of the lesions were fresh, while others were in the process of healing, so that the relations were not clear. On gross examination there were grave myocarditis, characterized by reddish, softened foci, irregular in size and shape, and large areas of necrosis in the renal cortex. It was important to determine whether these necroses were secondary to the circulatory failure, as thought by Hueper, or whether they were pri-

13. Collip, J. B.: *J. Biol. Chem.* **64**:485, 1925.

14. Collip, J. B.: *Medicine* **5**:1, 1926; *Am. J. Physiol.* **76**:472, 1926.

15. Hueper, W.: *Arch. Path.* **3**:14, 1927.

mary, and the circulatory failure secondary to the destructive lesions in the heart. This led to a study of the development of the lesions. In the rat, as in the dog, it was readily determined that preceding death the circulatory system was severely injured, with the skin surfaces becoming cyanosed and cold. In the terminal stages the tail especially showed cyanosis. Transudates were also found in the body cavities.

It is pertinent to call attention at this point to the report of Edwards and Irvine<sup>16</sup> on a dog given a fatal dose of hormone. Arrhythmia and a decrease in the heart rate appeared during the first twenty-four hours, but a severe fall in arterial pressure was not noted until the second twenty-four hours.

*Pathogenesis of Lesions Caused by the Hormone.*—A single large dose of hormone was injected into each of two rats (19 and 20, table 3). One kidney was removed for biopsy at twenty-four hours, and in it small areas of necrosis were found. At the end of forty-eight hours, necroses were present in the myocardium and in the other kidney. At this time there were neither general circulatory disturbances nor local changes, such as thromboses.

In neither these rats nor ones with larger necroses could we demonstrate thromboses of small vessels, except within the larger and older lesions, where they were secondary. In this connection, it is important to note that all animals were killed and the tissues immediately placed in the fixative. We are therefore of the opinion that the necroses are not malnutritional in origin, such as might result from general or local circulatory disturbances, but that they are toxic lesions. In these two animals and in the others of table 3, the kidneys, heart, pancreas, voluntary muscle, blood vessels, lungs, stomach, spleen, liver, suprarenal glands, thyroid gland and parathyroid glands were examined histologically. Only the first four organs named showed degenerative lesions. The skeletal system was not examined. A detailed study of the teeth is being made by Prof. Irving Schour of the Dental College of the University of Illinois, who will publish his findings in a separate report.

After the character of the twenty-four and forty-eight hour lesions caused by a single dose had been determined, the appearance of the heart and kidney after multiple injections became more intelligible. Whether the lesion calcified or not, solution of the necrotic parenchyma followed. In the heart, the solution was more rapid, and a vascularized area with a few round cells was the result. These were often perivascular in location. In the kidney, actively regenerating epithelium often was seen extending beneath the necrotic cells to form a new lining for the tubules. Injury to the myocardium and the kidney tended to run parallel, but after injection of repeated small doses (9 and 10) minute

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16. Edwards, D. J., and Page, Irvine: *Am. J. Physiol.* **76**:207, 1926.

vascularized scars were identified in the heart only. Possibly renal lesions had been present and had healed without scar formation. The necroses observed in the pancreas (3 and 14, table 3) and the muscle (6 and 14, table 3) are discussed in a separate paragraph.

*Effect of Inactivation on Toxicity of the Hormone.*—In table 4 are shown the effects of the inactivated hormone on the rat and on the dog. It is seen that inhibition of parathyroid proliferation is the only one of the three hormonal effects retained after inactivation. Hypercalcemia was not found and destructive lesions of the organs were not produced.

We have considered from the first the possibility that the preparations of hormone might contain toxic substances as impurities unrelated to the hormonal component, and that these might be of such a character as to produce the lesions. If such were the case, it would be necessary

TABLE 4.—*Injections of Inactivated Hormone*

Animal	Weight, Gm.	Dosage, Units and Times Injected	Duration of Experi- ment, Days	Blood Serum Calcium, Mg. per 100 Cc.	Necrosis	Calcifi- cation
1	120	50 (3×)	3	10.9	—	—
2	105	50 (3×)	3	10.7	—	—
3	115	50 (3×)	3	10.3	—	—
4 (dog)	14 Kg.	140 (3×)	4	11.8*	—	—
5 (dog)	19 Kg.	140 (3×)	2	11.2*	—	—
6	120	50 (3×)	3	10.6	—	—
7	135	50 (3×)	3	10.9	—	—
8	160	50 (3×)	3	10.8	—	—
9	120	50 (3×)	3	10.6	—	—

\* These are blood plasma calcium values.

to suppose that inactivation of the hormone also renders the toxic impurity inert. The absence of toxic lesions in the animals receiving inactivated hormone therefore strengthens the view that the lesions observed are caused by the parathyroid hormone and not by any associated impurity. The only determined effect of the inactivated hormone is the arrest of mitosis in the parathyroid cells (table 2). This has been discussed in the first division of the paper.

*Relationship of Necrosis and Calcification.*—Shelling,<sup>17</sup> in an extensive investigation of the effects on the rat of large doses of viosterol, observed destructive lesions of the soft tissues without calcification. He said: "Viosterol in very large doses causes a general toxic effect, since it may produce necrosis and inflammation without calcification." Viosterol then may act independently of the deposition of calcium to produce necrosis. We found this to be true for parathyroid hormone. In our hormone-treated rats we observed that necroses occurred, especially in the myocardium and kidney, without deposition of calcium

17. Shelling, D. H.: J. Biol. Chem. 96:241, 1932.

(table 3). We were especially desirous of determining the relationship of these two pathologic changes.

Rats (19 and 20, table 3) were given injections of single doses of hormone. At the end of forty-eight hours, widespread areas of necrosis were present in the renal cortex with a very slight amount of calcium here and there. Examination of the kidneys removed by unilateral nephrectomy at twenty-four hours showed beginning necrosis without any calcium. In three rats (9, 10 and 16, table 3<sup>18</sup>) there were necrotic foci in the heart without a trace of calcium. After multiple injections, early lesions without calcium and late ones with calcium may be seen in a single animal, as in rat 8, table 3.

All the observations made in the rat indicate that the necrosis occurs first, and that deposition of calcium in the degenerated areas follows.

In no animal have we seen calcification unassociated with a destructive lesion. The necrosis appears first (as early as eighteen hours, as actually determined in our experiments), and later the lesion may or may not become calcified. In rat 3 (table 3), healing of the renal lesions is shown. There are long stretches of tubules entirely devoid of epithelium and consisting merely of deposits of calcium between two walls of membrana propria. Elsewhere regenerating epithelium is actively extending beneath such deposits to reline and to reform the tubules. Some of the foci finally become patches of fibrosis, while elsewhere restoration to normal results.

In the rat we have not seen metastatic calcification, in the sense that it is a deposition of lime in tissues otherwise normal. It is probable that in the hypercalcemic rats there is an increased tendency for calcium to be deposited in the necrotic tissues.

*Production of Necrosis in Parenchymatous Organs by Calcium Gluconate.*—Collip<sup>14</sup> stated that lesions could not be produced by injections of calcium salts alone. We find that by intraperitoneal injections of calcium gluconate necroses may be produced in the myocardium and kidneys of the rat (6 to 11 inclusive, table 5). Necrosis was demonstrated as early as seven hours after a series of injections, while beginning calcification of the necrotic areas was seen at eight hours. The lesions appeared to be exactly like the early hormonal ones. The rats receiving these huge doses showed severe toxic symptoms, and only two of eleven lived the eight hour period.

*Local Toxic Action of Parathyroid Hormone (table 6).*—Before these experiments were made, necroses seen in both the pancreas and the abdominal muscles were associated by us with local action of the hormone injected intraperitoneally. This suspicion was confirmed by

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18. Animals 8, 9, 10 and 16 (table 3) are designated as 2, 3, 4 and 9, respectively, in table 2.

injection of the hormone directly into the tissues. In the kidney, a large necrotic focus was produced, not only by the hormone in the concentration used for intraperitoneal injection, but also when a 1:10 dilution of this was used. This was true for the para-thor-mone (rats 5 and 6) and also for the hormone prepared by the method of

TABLE 5.—*Injections of Calcium Gluconate*

Rat	Weight, Gm.	Dose (Cc.) of 10% Calcium Gluconate and Times Injected	Duration of Experiment	Blood Serum Calcium, Mg. per 100 Cc.	Necroses
1	145	1 (1×)	20 min.	....	.....
2	125	1 (2×)	80 min.	....	.....
3	180	1 (2×)	2 hr.	22.5*	.....
4	120	1 (4×)	6 hr.	....	.....
5	125	1 (4×)	6½ hr.	....	.....
6	135	1 (4×)	6¾ hr.	....	Kidney (minute foci)
7	140	1 (4×)	6¾ hr.	....	Kidney (minute foci)
8	140	1 (4×)	7¼ hr.	....	.....
9	150	1 (4×)	7½ hr.	....	Kidney (large foci); heart +
10	150	1 (4×)	8 hr.	22.5*	Kidney (large foci)†; heart +
11	150	1 (4×)	8 hr.	22.5*	Kidney (large foci); heart +

\* Determined in a specimen of pooled serum from rats 3, 10 and 11. Difficulty was experienced in obtaining sufficient blood for analysis after the repeated injections.

† Only one, which showed distinct deposits of calcium in the form of granules. In the kidney of rat 8 deposition of calcium had begun.

TABLE 6.—*Local Action of the Hormone*

Rat	Weight, Gm.	Dosage, Units*	Duration of Experiment, Days	Tissue Into Which Injection Was Made	Necroses at Site of Injection
1	140	2 L	2	Kidney	++
2	130	2 L	2	Kidney	++
3	150	2 L	1	Kidney, spleen, internal oblique muscle	Spleen, muscle and liver
4	125	2 L	1	Kidney, spleen, internal oblique muscle	Spleen, muscle and liver
5	135	0.2 L	1	Kidney	+
6	140	0.2 L	1	Kidney	++
7	170	0.2	1	Kidney	+
8	130	0.5% cresol	1	Kidney	+
9	250	2%	1	Kidney	++
10	250	0.2	1	Kidney	++
11	220	0.2% cresol	1	Kidney	—
12	220	2% cresol	1	Kidney	+

\* The dose was contained in 0.1 cc. of fluid.

Tweedy (rat 10), which contains no preservative. Acting on the assumption that para-thor-mone may contain a 0.2 per cent concentration of cresol as preservative, we injected cresol solutions of varying strengths directly into the kidney. With the higher concentration necrosis was produced, but with the more dilute solution no necrosis resulted. The necroses resulting from the direct contact of renal epithelium and hormone were the product of hormone and not of a preservative.



*The Mechanism of the Toxic Action of Parathyroid Hormone.*—Parathyroid hormone is not the only internal secretion known to act on the tissues to produce necrosis. Fleisher and Loeb<sup>19</sup> found that epinephrine is able to produce focal necroses in the myocardium of the rabbit. Later Johnson and Seibert<sup>20</sup> studied these destructive myocardial changes, but did not arrive at a satisfactory explanation.

Our experiments with parathyroid hormone have somewhat narrowed the problem of the manner of production of the lesions. They are not secondary to circulatory changes, either congestive heart failure or local thromboses. They are toxic lesions, which are usually associated with hypercalcemia. Since they may be produced by intraperitoneal injections of calcium gluconate, the obvious inference is that the calcium salts produce the lesions. When calcium gluconate as a 1 per cent solution was injected directly into the renal substance, no necrosis was seen about the hemorrhagic needle tract. The direct contact of calcium gluconate with parenchymal tissue appears not to produce the necrosis, but when it is injected in huge doses intraperitoneally, renal necrosis follows. The gluconate, like the parathyroid hormone and like viosterol, causes hypercalcemia. The hypercalcemia is likely associated with a disturbance in the relationship of ionized, nonionized, bound and unbound calcium both in the blood plasma and in the tissue lymph that bathes parenchymal cells. In the latter location, the changed calcium composition of the fluid so injures the cell that necrosis follows. The hormone when injected directly into the renal substances has a similar action.

#### CONCLUSIONS

Parathyroid hormone inhibits mitotic proliferation of the parathyroid gland. It does this in amounts insufficient to produce hypercalcemia and destructive lesions of parenchymatous organs.

The lesions produced by excessive doses of the hormone are toxic and primary. The circulatory failure which characterizes the fatal cases is secondary to the destructive lesions in the myocardium.

The hormone when brought into direct contact with parenchymatous cells produces lesions analogous to those produced after its absorption into the blood stream. Necroses are produced in the kidney and heart by intraperitoneal injection of calcium gluconate alone. It is probable that the hormone and calcium gluconate as well as viosterol injure parenchymal cells by disturbing the calcium components of the tissue fluids of the cells themselves.

There is no evidence of metastatic calcification in the rat in the sense that the calcium is deposited in tissue otherwise normal. In the

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19. Fleisher, M. S., and Loeb, L.: Arch. Int. Med. **3**:78, 1909.

20. Johnson, S., and Seibert, W. J.: Am. Heart J. **3**:279, 1928.

rat, local degenerations of the tissues are primary and precede calcification.

Amounts of hormone insufficient to produce effects demonstrable by the methods previously used successfully arrest mitotic proliferation of the parathyroid gland. Amounts not large enough to elevate the serum calcium may cause myocardial lesions.

Inactivated hormone, completely inert as determined by the usual method employed for testing activity, arrests parathyroid proliferation, but produces neither hypercalcemia nor destructive lesions.

# TRANSFUSION EXPERIMENTS WITH THE BLOOD OF LEUKEMIC CHICKENS

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AND

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PHILADELPHIA

Studies of avian tumors caused by filtrable agents have shown that the tumor cells produced by these agents behave like neoplastic cells (Rous<sup>1</sup>). Attention has been called to the similarity between avian sarcoma and avian leukosis.<sup>2</sup> This view is much strengthened by recent investigations<sup>3</sup> showing that avian leukosis, like avian sarcoma and unlike leukemia and tumors of mammals, may be transmitted by a cell-free agent, which passes bacteria-tight filters, resists freezing and thawing, and drying, and can be preserved by the addition of glycerin. However, direct evidence for the neoplastic character of the leukemic cells has hitherto been wanting. The differences in behavior between cell-free filtrate and cell-containing material transmitting leukosis might be explained by assuming that they are due to the position of the agent, largely intracellular in one instance, free in the other. Whether this agent is an ordinary virus or not, leukosis may be conceived of either as hyperplasia or as neoplasia of the myeloid and erythroblastic elements of the bone marrow.

Should the view be correct that leukosis is a neoplastic process caused by an ordinary virus, the immature blood cell may be expected to multiply and to retain transmissibility even after the disappearance of the causative agent. A similar phenomenon has been observed with mammalian tumors caused by infective agents.<sup>4</sup> Avian tumors caused by filtrable agents are, however, not known to be changeable into tumors that are transmissible only by material containing cells. Yet this

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From the Henry Phipps Institute, University of Pennsylvania.

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1. Rous, P.: *J. Exper. Med.* **18**:416, 1913.

2. Opie, E. L.: *Medicine* **7**:31, 1928.

3. Furth, J.: *J. Exper. Med.* **55**:465, 1932.

4. Fibiger, J.: *Overs. o. d. kong. Danske Videnskabernes Selsk. Forh.*, 1913, no. 1, p. 47. Jensen, C. O.: *Den kongelige Veterinaer-og Landbohøjskoles Aarskrift*, Copenhagen, 1918, p. 91. Both references are quoted from Krebs, C.; Rask-Nielsen, H. C., and Wagner, A.: *Acta radiol.*, supp. 10, 1930, p. 1.

would frequently occur were these agents viruses characterized by the capacity to produce strong immunity in the animals affected. A fowl immune to the filtrable agent of leukosis was inoculated with leukemic cells.<sup>5</sup> We looked for an inactivation of the agent and a transformation of avian leukosis transmissible by a filtrable agent into avian leukosis transmissible only by leukemic cells. This supposition was not substantiated; the cell-free plasma of a fowl thus treated readily transmitted the disease.<sup>5</sup>

The experiments to be described were made in an attempt to ascertain whether leukemic cells introduced into the circulation in large numbers are capable of autonomous growth in the body of a susceptible host.

Minot and Isaacs<sup>6</sup> transfused blood of a patient with lymphatic leukemia into one with aleukemic lymphoma and noted almost complete removal of the immature lymphocytes from the circulation within thirty minutes. Experiments with transmissible lymphoid leukemia of mice<sup>7</sup> indicate that leukemic lymphocytes introduced into the circulation of healthy mice lodge and multiply mainly in the lymphoid tissues and secondarily invade the circulating blood. Lymphocytes from mice with malignant aleukemic lymphadenosis,<sup>8</sup> when injected into the veins of healthy mice, likewise multiply in lymphoid tissues and infiltrate many organs, but do not invade the blood stream to any considerable extent.

Leukosis of fowls differs from lymphoid leukemia of guinea-pigs and mice<sup>9</sup> in that it is transmissible by a cell-free filtrable agent. Since mammalian lymphoid leukemia may be reproducible by cells, it is again pertinent to inquire whether avian leukosis may result from the multiplication of transfused leukemic cells.

#### FIRST SERIES OF TRANSFUSION EXPERIMENTS

From 15 to 35 cc. of the blood of fowls with severe myeloid leukemia was transfused into each of seven young chickens after a like or somewhat smaller amount of blood had been removed from the circulation of the recipient. In five of these fowls the transfused immature myeloid cells were rapidly removed from the circulation, but in two of them they multiplied rapidly, causing death within three days with the blood picture of leukemia. Figure 1 *A* and *B*, table 1 and the history of four fowls illustrate these observations.

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5. Furth, J.: Immunity Phenomena in Transmissible Leucosis of Fowls, *Proc. Soc. Exper. Biol. & Med.* **29**:1236, 1932.

6. Minot, G. R., and Isaacs, R.: *J. A. M. A.* **84**:1713, 1925.

7. Furth, J., and Strumia, M.: *J. Exper. Med.* **53**:715, 1931.

8. Seibold, H. R.; Rathbone, R. R., and Furth, J.: *Proc. Soc. Exper. Biol. & Med.* **29**:629, 1932.

9. Tio Tjwan Gie: *Over leukaemie bij dieren*, Amsterdam, 1927. Korteweg, R.: *Ztschr. f. Krebsforsch.* **29**:455, 1929. MacDowell, E. C., and Richter, M. N.: *Science* **74**:605, 1931.

*Notes to Table 1.*—The column headed "Primitive Myeloid Cells" refers to the cells illustrated in figure 1 *A*; the majority of these are primitive mononuclear cells, or myeloblasts according to Ellermann. Many similar cells contained lobed nuclei or several nuclei; these, called poikilonuclear cells by Ellermann and Rieder cells by others, are included in this column. A few such cells containing purple granules (promyelocytes) were also counted with these. The myeloid cells of this fowl will be more fully described in a later report. The column headed "Polychrome Red Cells" includes both polychrome erythroblasts and erythrocytes. The percentage of normal leukocytes decreased following the transfusion, but their absolute number increased somewhat. The bulk of the increase in white cells is referable to an increase in primitive large mononuclear leukocytes.

TABLE 1.—*Blood Changes in Fowls That Had Received a Transfusion of Leukemic Blood*

Fowl	Time of Examination with Relation to Transfusion	Hemo- globin, (Sahli), per Cent	Red Cell Count, Thou- sands	White Cell Count, Thou- sands	Poly- chrome Red Cells, Thou- sands	Baso- phil Tryth- ro- blasts, Thou- sands	Poly- morpho- nuclear Leuko- cytes, per Cent	Lym- pho- cytes, per Cent	Mono- cytes, per Cent	Mast Cells, per Cent	Primi- tive Myeloid Cells, per Cent
1627	Before.....	46	2,530	30	3	0	18	69	6	6	1
	30 min. after...	41	1,570	189	54	4	2	14	1	1	82
	1 day after....	34	1,870	265	21	0	2	5	1	1	91
	2 days after...	32	1,405	265	29	0	2	6	1	1	90
	3 days after...	38	1,645	585	29	0	2	6	0.5	0.5	91
1493	Before.. .....	59	2,355	27.5	0	0	30	60	4	6	0
	30 min. after...	35	1,560	365	58	4	7	11	0.5	0.5	81
	3 days after...	27	1,240	910	15	0	1.5	1.5	0.5	0	92.5
	(In vitro mix- ture of donor's and recipient's blood [1:3])	36	1,875	912	110	10	4.5	3.5	0.5	0.5	91
1106	Before.....	56	2,890	48.5	0	0	32	61	6	1	0
	30 min. after...	37	1,500	173	107	20	21	11	4	1	62
	1 day after....	34	1,585	153	29	2	22	20	2	2	54
	2 days after...	34	1,505	230	27	0	20.5	15	3	0.5	61
	4 days after...	32	1,305	107	51	2	22	40	1	3	34
	6 days after...	39	1,710	91	0	0	30	59	9	1	0
	8 days after...	34	1,735	81	0	0	15	68	5	2	0
	11 days after...	53	2,500	48	0	0	18	76	5	1	0
	14 days after...	55	3,000	43	0	0	29	64	5	2	0

*Experiment 1.*—Thirty cubic centimeters of blood of a fowl with severe myeloid leukemia was transfused into a chicken weighing 870 Gm. The white cell count of the donor was 975,000; the red cell count, 1,030,000, and the hemoglobin content, 18 per cent (Sahli). The blood counts of the recipient before and after the transfusion are recorded in table 1. (See also figure 1 *A*.) There was a rapid, progressive rise of the immature myeloid cells in the circulation, causing death of the fowl three days after the transfusion. In blood smears taken before death, large numbers of mitotic figures were seen (fig. 2 *A*). At postmortem examination, the liver and the spleen were somewhat enlarged; their pale grayish color suggested an invasion by white cells. Microscopically, stasis of immature myeloid cells was observed in many organs, notably in the lung (fig. 2 *C*), spleen (fig. 3 *E*), liver (fig. 3 *A*), kidney (fig. 2 *E*) and adrenal gland. In the liver there

were, also, numerous large tumor-like foci formed apparently by multiplying myeloid cells. Most of the bone marrow was composed of fat (fig. 2*B*); only a few small areas were seen that showed the character of leukotic growth, namely, multiplication of primitive cells with little or no maturation.

*Experiment 2.*—Twenty cubic centimeters of leukemic blood was transfused into a fowl weighing 750 Gm., after the removal of 15 cc. of its own blood. The leukemic blood had a white cell count of 915,000, a red cell count of 540,000 and a hemoglobin content of 14 per cent (Sahli). As in the preceding fowl, the transfusion was followed by a progressive rise in the number of circulating immature myeloid cells (table 1), terminating in death three days after the transfusion. The gross and microscopic appearances of the organs of this fowl were essentially the same as those described in experiment 1.

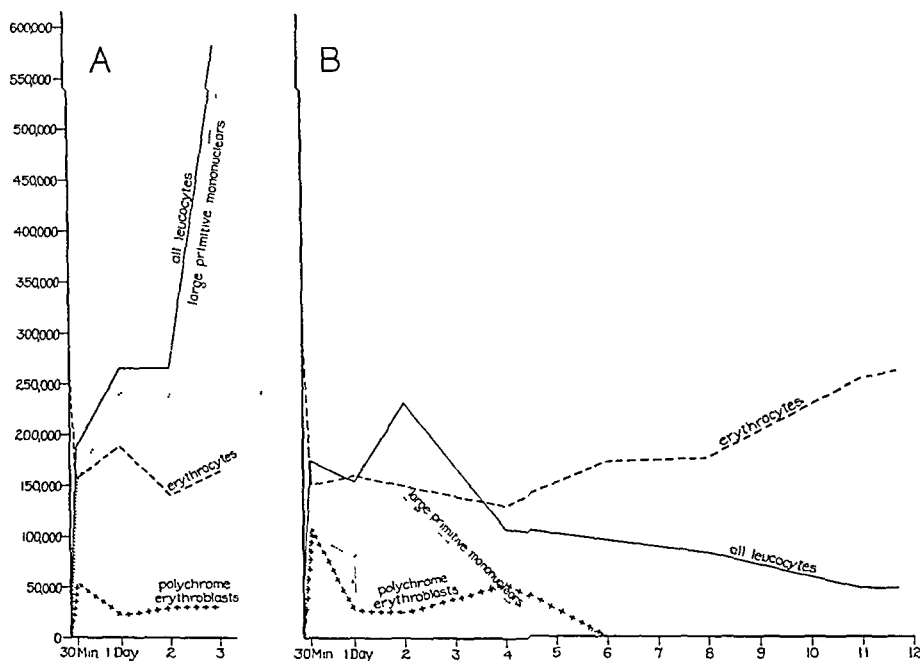


Fig. 1.—*A*, chart showing the changes in the number of blood cells of a fowl after it had received a transfusion of 30 cc. of leukemic blood. The fowl died of leukemia three days after the transfusion. *B*, chart showing the changes in the number of blood cells of a fowl that had received a transfusion of 30 cc. of leukemic blood. The transfused cells rapidly disappeared from the circulation.

In these two experiments, massive transfusion of leukemic blood into two healthy fowls resulted in leukemia fatal on the third day after the transfusion. The effect of the transfusion on the number of circulating leukocytes is shown in table 2. The figures in table 2 do not give exact information as to the rate of multiplication of the transfused primitive myeloid cells, mainly because these cells are retained in large numbers in the capillaries of many organs, and data on the number of immature leukocytes thus removed from the circulation and on the rate of their multiplication in the capillary bed are wanting. However, large numbers of mitotic figures seen in the circulating blood as well as in the

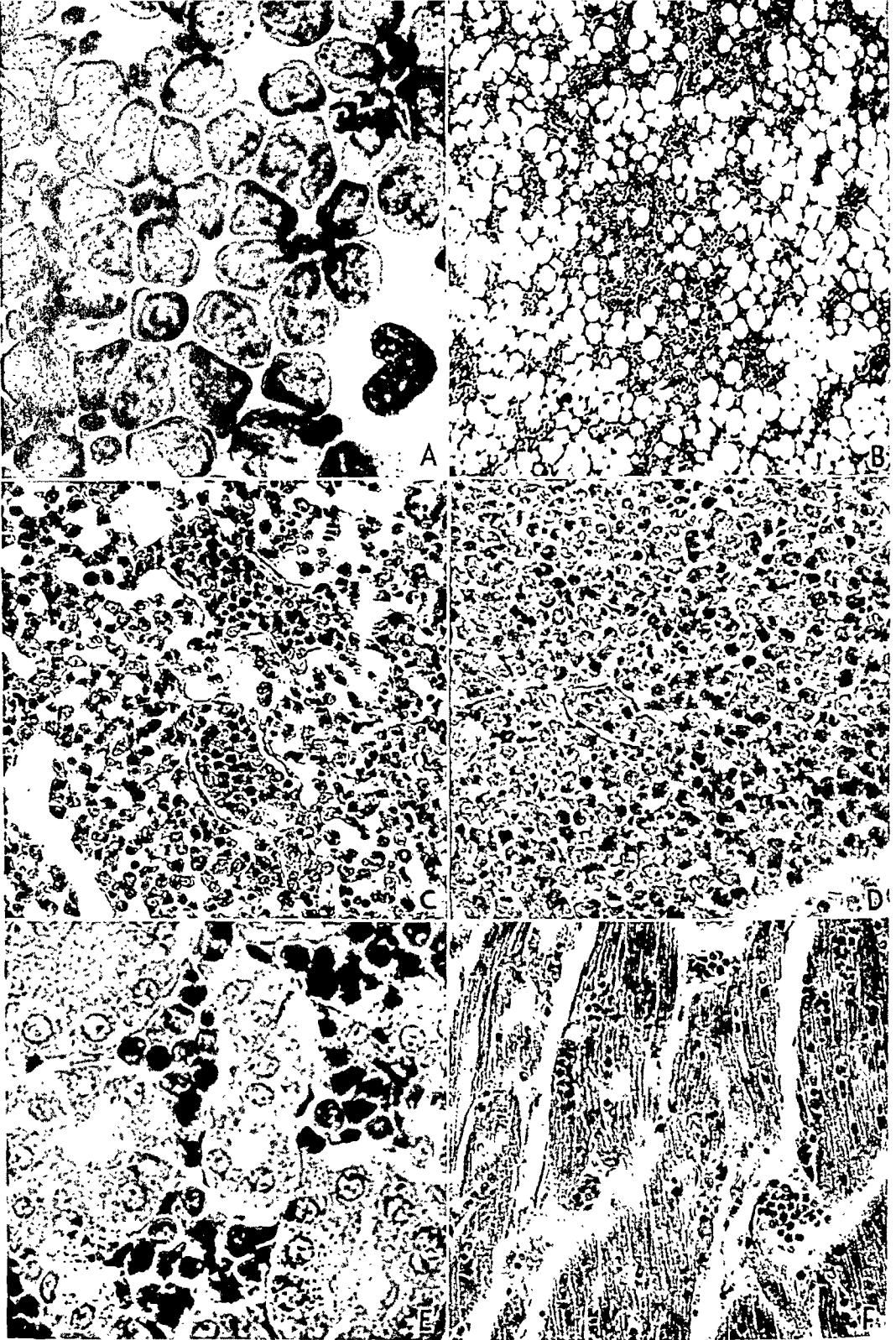


FIGURE 2

Photomicrographs are shown of material from fowls that died about three days after receiving transfusion of leukemic blood. The blood smear is stained with Wright and Kiernsa's solution, and the slides, with hematoxylin and eosin. The magnifications given are only approximate. *A* shows a blood smear with four mitotic figures in a field ( $\times 900$ ); *B*, femoral marrow containing abundant fat ( $\times 25$ ); *C*, capillaries of the lung distended with myeloid cells ( $\times 250$ ); *D*, an area of the lung, its capillaries filled with myeloid cells, many of them in mitotic division ( $\times 300$ ); *E*, mild stasis of myeloid cells in the kidney ( $\times 500$ ), and *F*, mild stasis of myeloid cells in the heart muscle ( $\times 200$ ).

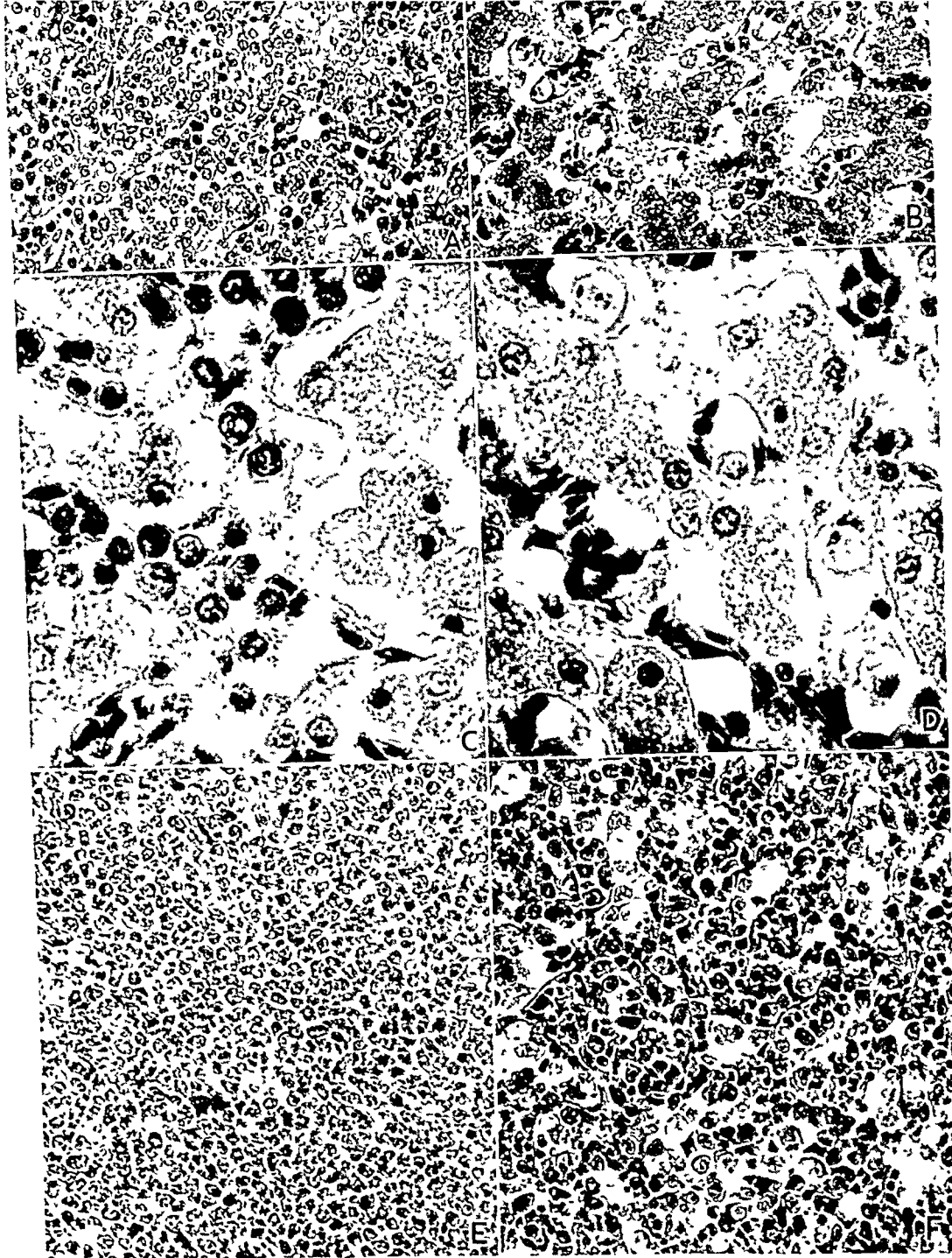


FIGURE 3

Photomicrographs of liver and spleen of fowls dying with leukemia about three days after they had received a transfusion of leukemic blood are contrasted with those of fowls in which there was a rapid removal of the transfused leukemic cells. The slides were stained with hematoxylin and eosin. The magnifications given are only approximate. *A* shows liver from a fowl dying with leukemia after transfusion ( $\times 250$ ). Note the accumulation of large numbers of apparently viable cells in the capillaries, showing practically no tendency of the host to remove them. *B* shows liver from a fowl in which the transfused leukemic cells disappeared from the circulation ( $\times 250$ ). Intact leukemic cells are scant in the capillaries, while the Kupffer cells show extensive phagocytic activity. *C* is a higher magnification ( $\times 600$ ) of the slide shown in *A*, and *D*, a higher magnification ( $\times 600$ ) of the slide shown in *B*. *E* pictures the spleen of a fowl dying with leukemia after transfusion ( $\times 250$ ). The pulp is densely crowded with apparently viable leukemic cells; little, if any, phagocytic activity is seen. *F* shows the spleen of a fowl in which the transfused leukemic cells disappeared from the circulation ( $\times 250$ ). Mononuclear phagocytes engulfing cells or cellular debris are abundant in the field.



capillaries may be taken as an indication that the transfused cells underwent rapid multiplication (fig. 2 *A* and *D*). It is hardly conceivable that the normal marrow of a fowl would be capable of producing leukocytes in such numbers in such a short time. Moreover, this possibility is excluded by an examination of the marrow after death, which showed abundant fat and little new formation of myeloid cells (fig. 2 *B*). Growth within blood vessels, an abnormal site for myeloblast formation, had taken place apparently throughout the vascular bed; extravascular growth, localized mainly in the liver and the bone marrow, was relatively slight.

Experiment 3 is described as an example of five experiments in which transfusion of leukemic blood was followed within a few days by complete disappearance of the immature blood cells from the recipient's circulation. Three of the five fowls showed no further change, but in one erythroleukosis developed forty-seven days after the transfusion.

TABLE 2.—*Effect of a Transfusion of Leukemic Blood on the Leukocyte Counts of Healthy Fowls*

Fowl	Count Before Transfusion	30 Minutes After Transfusion	3 Days After Transfusion
1623.....	30,000	189,000	585,000
1493.....	27,500	365,000	910,000

*Experiment 3.*—Thirty-five cubic centimeters of leukemic blood was transfused into a fowl after the removal of 30 cc. of its blood. The white cell count of the leukemic blood was 1,050,000; the red cell count, 750,000, and the hemoglobin content, 19 per cent (Sahli). The blood counts and differential counts of the recipient made before and after the transfusion are recorded in table 1. (See also figure 1 *B*.) The highest number of immature blood cells in the circulation was observed two days after the transfusion (white cell count, 230,000); four days after the transfusion, their number had decreased considerably, and two days later there was merely a leukocytosis due to an increase of normal lymphocytes, polymorphonuclear leukocytes and monocytes. Subsequent examinations of the blood during a period of five months and the postmortem examination showed neither anemia nor leukemia.

As another example of the rapid elimination of immature leukocytes from the circulation of healthy fowls, a fowl may be cited the white cell count of which was 17,000 before transfusion, 218,000 one-half hour after transfusion and 34,500 three days after transfusion.

#### SECOND SERIES OF TRANSFUSION EXPERIMENTS

About a year after the experiments reported in the foregoing paragraphs had been completed, eight chickens, weighing from 550 to 750 Gm., were given transfusions of leukemic blood in the manner described. These experiments were undertaken to throw light on the mode of removal of the transfused cells. It is remarkable that in the

case of only one of the eight chickens was there a drop in the high leukocyte count caused by the mechanical effect of transfusion; the rest died with the blood picture of leukemia from two to three and a half days after the transfusion.

The view that the rise in the blood counts of these seven fowls following the transfusion (table 3) indicates multiplication of these cells in the recipients was strengthened by the observation of numerous mitotic figures in smears as well as by that of the absence of conspicuous myeloid hyperplasia of the marrow. Nevertheless, it is evident from the figures given in table 3 that the death of the fowls from two to three and

TABLE 3.—*Transfusion Experiments (Second Series)*

Recipi- ent	Volume of Blood, Cc.		Donor's Blood Count, Thousands		Recipient's Blood Count, Thousands						Comment
					Before Transfusion		½ to 1½ Hr. After Transfusion		2 Days After Transfusion		
	Re- moved	In- jected	White Blood Cells	Red Blood Cells	White Blood Cells	Red Blood Cells	White Blood Cells	Red Blood Cells	White Blood Cells	Red Blood Cells	
2332	20	40	995	1,150	16.7	1,822	405	1,310	850	1,095	Died 2½ days after transfusion
2330	15	25	1,120	895	35	1,950	420	1,395	740	1,025	Died 3½ days after transfusion
2333	17	25	1,120	895	29.5	2,430	645	1,540	1,390	1,150	Died 2 days after transfusion
2346	8	10	1,390	1,150	43	2,650	208	2,185	400	1,410	Died 2½ days after transfusion
2347	10	10	1,390	1,150	39.5	2,820	120	2,455	204	1,500	Died 2 days after transfusion
2348	10	20	690	1,180	31.5	2,005	119	1,820	182	1,720	Died 3 days after transfusion
2349	10	20	690	1,180	19	2,920	74	1,870	153	1,485	Died 3½ days after transfusion
2327	20	30	495	1,412	45	2,505	230	1,745	81.5	1,330	Active; killed 3 days after transfusion

a half days after the transfusion cannot be correlated with the height of their white cell counts. These counts from two to three days after the transfusion were about double those obtained from one-half to one and a half hours after the transfusion, whether increasing from 74,000 to 153,000 or from 645,000 to 1,390,000. What characterizes this group is not the height of the white cell counts, but their conspicuous rise in two and three days after the transfusion. On the other hand, fowls in which there was a drop in the number of white cells three days after the transfusion (fowl 2327 of series 2 and five fowls of series 1) appeared active, and if permitted to live, recovered from the immediate effects of the transfusion.

It is a matter of conjecture why most of the fowls of the first series resisted the transfusion, whereas most of the fowls of the second series succumbed to it. Increased virulence of the leukemic cells, selection of

somewhat younger birds or improved technic may be responsible for this difference.

#### COMMENT ON THE TRANSFUSION EXPERIMENTS

When large amounts of leukemic blood are transfused into fowls, either the white cells introduced into the circulation continue to increase in number within the vascular bed and the fowls die with the blood picture of leukemia from two to four days after the transfusion, or most of the transfused cells disappear from the circulation within three days, and the blood picture gradually returns to normal. Resistance and susceptibility in these instances are explained with most probability by an assumption of genetic differences similar to those that govern the fate of tumor grafts.

The experiments described indicate that leukemic chicken cells introduced into susceptible hosts are capable of autonomous growth. The behavior of lymphocytes causing lymphoid leukosis of mice<sup>10</sup> suggested a similar conclusion. Accordingly, it may be supposed that leukemic cells from either animal would multiply *in vitro* under favorable conditions as do cancer cells. Contrary to these suggestions, investigators studying the behavior of leukemic cells of man in tissue cultures describe, instead of multiplication, maturation.

#### ANATOMIC CHANGES IN FOWLS THAT RECEIVED TRANSFUSIONS OF LEUKEMIC BLOOD

In order to determine the site of retention and subsequent disposal of the introduced cells in resistant fowls, seven such chickens that had received transfusions of leukemic blood were killed at different intervals after the transfusion, and their organs examined microscopically.

There are numerous studies on the mechanism of the disposal of foreign particles introduced into the circulation as well as of cell debris originating within the body. For a review of the literature see Drinker and Shaw.<sup>11</sup>

Two fowls were studied one and a half hours after they had received the transfusion of leukemic blood. The white cell count rose in one to 350,000, as a result of the transfusion, and in the other, to 245,000. One fowl, examined twenty-four hours after it had received the transfusion of leukemic blood, had a white cell count of 260,000 one and a half hours after the transfusion and one of 220,000 before death. Two fowls were examined three days after they had received the transfusion. The white cell count was 218,000 one-half hour after the transfusion and 34,500 before death, in one, and in the other, 230,000 and 81,500, respectively. In both fowls the blood smears taken before death showed many primitive mononuclear cells (myeloblasts). Two other fowls that died of leukemia three days after they had received a transfusion of leukemic blood have been described (pp. 662 and 663). Two fowls were studied six days after they had received a transfusion, one

10. Furth and Strumia.<sup>7</sup> Seibold, Rathbone and Furth.<sup>8</sup>

11. Drinker, C. K., and Shaw, L. A.: *J. Exper. Med.* **33**:77, 1921.

of 12 and the other of 25 cc. of leukemic blood. In smears taken before death there were practically no immature myeloid cells, but a few erythroblasts had made their appearance, suggesting the onset of erythroleukosis caused by the transfused leukemic material.

It is evident from the microscopic appearance of the organs of these fowls that a considerable proportion of transfused leukemic cells was retained in the pulp of the spleen and in the capillaries of several organs, notably the lung and the liver. The part played by the spleen was especially prominent; the pulp appeared to be filled to capacity thirty minutes after the transfusion and thereafter for about six days. The behavior of the bone marrow was very different; here sinusoidal capillaries were filled with erythrocytes, but among them only a few primitive myeloid cells of the type transfused were seen.

In the fowl examined twenty-four hours after transfusion there was phagocytosis of cellular debris by the Kupffer cells, but the majority of the primitive myeloid cells filling the pulp of the spleen and accumulating in the capillaries of the liver appeared viable. Myeloid cells were much more numerous in the sinusoidal capillaries of the bone marrow in this fowl than in those examined one-half hour after the transfusion, and many so-called intersinusoidal capillaries contained such cells almost exclusively.

In the fowls examined three days after transfusion, the appearance of the larger blood vessels indicated that most of the immature white cells had been removed from the circulation. Stasis of myeloid cells in the pulp of the spleen and in the capillaries of the liver and of the lung was less extensive than in the fowl just described; phagocytosis by mononuclear leukocytes, on the other hand, was more conspicuous (fig. 3 *B*, *D* and *F*). In the bone marrow, primitive mononuclear cells were abundant; among them were numerous myelocytes. Most of the erythrocytic capillaries were collapsed. Their lining could not be clearly distinguished, and for this reason it could not be determined with certainty whether this accumulation of myeloid cells had occurred only in extravascular tissues. It likewise remains a matter of conjecture whether the increase in the number of primitive nongranular myeloid cells was due to a slow migration of transfused myeloid cells to the marrow, or to a myeloid hyperplasia of the marrow, or to both.

In the fowls examined six days after the transfusion the Kupffer cells showed phagocytosis. The splenic pulp was filled with cells showing karyorrhexis and pyknosis, but here phagocytosis by mononuclear leukocytes was less pronounced, suggesting that lysis may be one of the means of disposal of dead cells.<sup>12</sup> The bone marrow showed mild non-specific hyperplasia of all its elements.

12. Strauss, A.: Beitr. z. path. Anat. u. z. allg. Path. **85**:251, 1930.

## COMPARISON OF ANATOMIC CHANGES IN SUSCEPTIBLE AND RESISTANT FOWLS THAT RECEIVED TRANSFUSIONS OF LEUKEMIC BLOOD

In a comparison of the microscopic appearances of the organs of susceptible and resistant fowls three days after the transfusion of leukemic blood, the differences in their reaction to the foreign leukemic cells are clearly seen. In susceptible fowls, the capillaries of the lung are filled with leukocytes, causing in some areas almost complete atelectasis. The pulp of the spleen, too, is filled with leukocytes, and there are numerous mitotic figures among these cells in both the lung and the spleen. In the capillaries of several other organs, e. g., the liver, there is slight to moderate stasis of the leukemic cells. In resistant fowls, on the other hand, there are relatively few leukemic cells in the capillaries of these organs, and large mononuclear phagocytes containing cells or cellular debris are abundant in the liver and spleen. There is slight, if any, phagocytosis in the lung, suggesting that the cells retained in the pulmonary capillaries immediately after transfusion may be subsequently released and disposed of in the spleen and liver as described by Drinker and Shaw.<sup>11</sup>

## SUMMARY AND CONCLUSIONS

Of fifteen fowls into which leukemic blood had been transfused, nine died of leukemia from two to three and one-half days after the transfusion. The fatal leukemia in these instances was associated with multiplication of the transfused cells. Thus, when immature myeloid cells of the fowl have been stimulated by the filtrable agent of leukosis, they assume the character of tumor cells and are capable of autonomous growth.

A considerable proportion of the transfused cells was rapidly removed from the circulation in all the fowls. The spleen and the capillaries of several organs, mainly those of the liver and the lung, are active in performing this function. The bone marrow plays little part in the removal of the immature myeloid cells. In susceptible fowls, the cells retained multiply in these sites; in resistant fowls, they are disposed of by mononuclear phagocytes of the liver and spleen.

# TRUNCUS ARTERIOSUS COMMUNIS PERSISTENS

## CRITERIA FOR IDENTIFICATION OF THE COMMON ARTERIAL TRUNK, WITH REPORT OF A CASE WITH FOUR SEMILUNAR CUSPS

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Among the rare congenital defects of the heart is persistence of the primitive common arterial trunk. This is one of the most interesting of anomalies from a developmental point of view, as it involves a failure of certain of the primary septums, those of the arterial trunk and cardiac bulb. Hence it dates from a very early stage of cardiac development. Many of the cases reported under this heading are really examples of other defects. In spite of the increased appreciation of the embryologic basis of the anomaly, opinion varies widely as to which cases should be included in this group. Herxheimer<sup>1</sup> accepted forty-three cases. Abbott<sup>2</sup> recognized twenty-three cases, fourteen of which she analyzed and classed as instances of the "complete" defect. Recent critical reviews in the German literature have rejected many of these examples. Shapiro<sup>3</sup> recently stated that he knew of but two cases that fulfilled all requirements for identification of the defect.

Because of the confusion that prevails, it seems advisable to review the criteria for identification of the anomaly in the light of the newer embryologic knowledge. These criteria will be applied to an example of partial (almost complete) persistence of the common arterial trunk, one of the five reported cases in which four semilunar cusps have been found.

### EMBRYOLOGIC AND ANATOMIC FACTORS

Detailed reviews of the embryologic development of the heart are available in the works of Tandler,<sup>4</sup> Abbott,<sup>5</sup> Mönckeberg<sup>6</sup> and others.

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1. Herxheimer, Gotthold: *Missbildungen des Herzens und der Grossen Gefässe*, in Schwalbe: *Die Morphologie der Missbildungen des Menschen und der Tiere*, Jena, Gustav Fischer, 1910, pt. 3, no. 3, sect. 2, p. 427.

2. Abbott, M. E.: *Congenital Cardiac Disease*, in Osler and McCrae: *Modern Medicine*, Philadelphia, Lea & Febiger, 1927, p. 612.

3. Shapiro, P. F.: *Arch. Path.* **10**:671, 1930.

4. Tandler, Julius: *Anatomie des Herzens*, in Bardelben: *Handbuch der Anatomie des Menschen*, Jena, Gustav Fischer, 1913, vol. 3, pt. 1, p. 1.

5. Abbott, M. E., and Shanley, Eleanor: *Internat. A. M. Museums Bull.* **8**: 188, 1922. Abbott.<sup>2</sup>

6. Mönckeberg, J. G.: *Die Missbildungen des Herzens*, in Henke and Lubarsch: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol. 2, p. 1.

Only those features of special interest for the interpretation of the anomaly under consideration will be considered here.

The normal division of the primitive tubular arterial trunk and cardiac bulb occurs in the early weeks of development. Three elements aid in the division. These are the two proximal swellings of the bulb, which assist in forming the interventricular septum; the four distal swellings of the bulb, which form the distal bulb septum, and from the lower ends of which the semilunar cusps are derived, and the aortic-pulmonary septum, which completes the division of the trunk. Thus in an early stage of development the systemic and pulmonic blood streams are separated, except for the communications through the foramen ovale and ductus arteriosus, which normally become closed only after birth.

*Aortic-Pulmonary Septum.*—It is generally held that the aortic-pulmonary septum is first demarcated distally by the spurs between the fourth and sixth pairs of aortic arches. The left member of the fourth pair is destined to form the arch of the aorta and its branches, except the right subclavian artery, which develops from the right fourth arch. From the sixth pair come the right and left pulmonary arteries, and from the left, the ductus arteriosus. Fusion of the paired spurs and caudal extension of the resulting septum divide the primitive trunk into the aorta and the pulmonary artery. The septum bisects and grows through the larger opposed (lateral) pair of distal swellings, which fuse near their midpoints. Thus three swellings are contributed to each arterial ostium, as the anlagen for the semilunar cusps. The caudal end of the septum then joins with the proximal bulb septum to complete the separation of the two arterial cones and of the ventricles in which they become incorporated.

Accounts differ as to the manner of development of this septum. Mönckeberg,<sup>6</sup> following Born and Broman, held that it develops from two longitudinal ridges, or swellings, which come to separate the two blood streams by fusion of their margins and by caudal extension. Tandler's<sup>4</sup> view, supported by Wirtinger's<sup>7</sup> observations, is that the septum proceeds caudally as an arched membrane, sending ahead longitudinal ridges as forerunners of its course. Spitzer's<sup>8</sup> theory is that fusion of the two spurs and proximal migration are due to two factors. The increased flow of blood, due to opening of the pulmonary capillary bed, leads to a widening and shortening of the primitive trunk, causing a relative descent. The increasing blood pressure on the two surfaces of the septum causes a further centripetal migration, in which the forerun-

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7. Wirtinger, cited by Feller, A.: *Virchows Arch. f. path. Anat.* **279**:869, 1931.

8. Spitzer, Alexander: *Virchows Arch. f. path. Anat.* **243**:81, 1923.

ners descend ahead of the arched septum. On histologic grounds, Tandler<sup>4</sup> believed that the aortic-pulmonary septum grows through the line of fusion of the distal bulb swellings, thus forming the septum of the extracardiac portion of the bulb, as well as of the trunk.

*Septum of the Proximal (Cardiac) Bulb.*—The complex upper part of the interventricular septum is formed by a fusion of the primitive ventricular septum with the two proximal swellings of the bulb and with the anterior endocardial cushion of the atrioventricular ostium (Tandler<sup>4</sup>). It is difficult to identify the different elements in the definitive state. Spitzer<sup>8</sup> concluded from a study of the model of the heart of a 14.5 mm. human embryo, prepared by Tandler, that the course of the muscle structures constituting the crista aorticopulmonalis and the anterior tricuspid ledge indicates derivation from the bulb swellings and the anterior endocardial cushion. The relations of these structures to the distal bulb swellings of the base of the arterial trunk and to the anterior tricuspid leaflet indicate an intimate fusion rather than a juxtaposition of the elements derived from the bulb and the cushion.

The point of closure of the interventricular septum is in the anterior part of the membranous septum. A simple interventricular foramen associated with a failure of union of the elements named is located immediately beneath the aortic ostium, which is frequently shifted to the right, in the position of the "rider" aorta. With defects involving only the posterior part of the anterior septum (Rokitansky), the opening stands above a Y-shaped muscle ledge on the right wall of the septum. This is part of the crista and ledge referred to in the foregoing paragraph, and will be described later. The opening is bordered anteriorly by the remnant of the anterior swelling and a forerunner of the primitive ventricular septum; posteriorly, by the rest of the membranous septum from the anterior endocardial cushion, and behind that, by the posterior muscular septum from the cushion and the posterior forerunner of the interventricular septum. Its lower margin, between the divergent limbs of the Y, is formed by fused remnants of the anterior swelling, the posterior swelling and a protuberance of the right anterior endocardial cushion. Except with more extreme septal defects, the primitive interventricular septum does not border the opening.

*Semilunar Cusps and Coronary Arteries.*—For an analysis of cardiac defects a study of the relationships of the semilunar cusps to adjacent structures and to the coronary arteries is essential. If normally placed, the coronary ostia identify the cusps. In identifying and naming the coronaries, however, one must take into consideration the type of the cardiac defect. Spitzer<sup>8</sup> has shown that with extreme detorsion one



coronary stem may take over or "adopt" many of the branches normally derived from the other. Thus, the apparent left coronary may be the right and vice versa. Even in otherwise normal hearts, variations of origin and course are common. Minor shifts of the ostia are frequent. Occasionally one ostium is shifted above the commissure between its cusp and that of the other coronary, so that the two may appear to arise from one sinus. Less frequently only one coronary stem may be present and may send branches to the field normally supplied by the other. With absence of the main stem, two ostia may be present in a single sinus. A rarer anomaly is the presence of only one aortic coronary, the other, usually the left, originating from a sinus of the pulmonary artery. With such variability, it is obvious why the coronary arteries identify the cusps only when found in the expected positions.

Little is known of the early development of the coronary arteries. From their studies of rabbit embryos, Martin<sup>9</sup> and Lewis<sup>10</sup> both concluded that the anlagen are recognizable before the division of the bulb. From their normal relationships it is obvious that they develop in relation to the larger (lateral) pair of distal bulb swellings. Obviously, too, their sites should be shifted toward the junctions of these swellings with the swelling for the future noncoronary aortic cusp. Thus, before rotation, the left coronary should originate near the anterior half of the right swelling, and the right, from the corresponding part of the left swelling, opposite. Normal torsion of 180 degrees would bring swellings and coronaries into their final normal relationships. After fusion and bisection of the lateral swellings, the anterior (pulmonic) ostium has three cusps, anterior and left and right posterior; the posterior (aortic ostium has the same number, posterior (noncoronary) and left and right anterior. If normally placed, the left coronary rises behind the left anterior, the right behind the right anterior, aortic cusp.

On theoretical grounds one would demand four semilunar cusps for the positive identification of a persistent common trunk. Gierke,<sup>11</sup> Wirth,<sup>12</sup> Pietzch<sup>13</sup> and Hülse<sup>14</sup> have emphasized this point, while admitting that anomalies of number of swellings may occur as a primary anomaly. Simonds<sup>15</sup> found 209 recorded cases of alteration in number of semilunar cusps, forty-three in 15,666 autopsies. Many of these were

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9. Martin, Henri: *Compt. rend. Soc. de biol.* **6**:83, 1894.

10. Lewis, F. T.: *Anat. Anz.* **25**:261, 1904.

11. Gierke, Edgar: *Charité-Ann.* **32**:299, 1908.

12. Wirth, A.: *Ein Fall von totaler Persistenz des Truncus arteriosus communis*, Diss., Giessen, 1912; cited by Mönckeberg.<sup>6</sup>

13. Pietzch, Johannes: *Ueber zwei Fälle von Atresia ostii aortae congenita*, Diss., Freiburg; cited by Mönckeberg.<sup>6</sup>

14. Hülse, Walter: *Virchows Arch. f. path. Anat.* **225**:16, 1918.

15. Simonds, J. P.: *Am. J. M. Sc.* **166**:584, 1923.

in otherwise normal hearts. Variations were more frequent in the pulmonic than in the aortic ostium, and reduction was commoner than increase. Simonds offered three possible explanations of reduction. Antenatal or postnatal fusion of two cusps is suggested by finding one exceptionally large cusp with a ridge at the base of its sinus. A shift in the position of the aortic-pulmonary and distal bulb septum may be responsible for some of the two-cusped valves found in stenosed vessels. One of the four swellings may be absent or may regress at an early period. Four-cusp valves are infrequent; Simonds found reports of but five cases of four-cusped aortic valves, four of them in normal hearts. It is difficult to explain the acquisition of a perfectly formed, though often small, extra cusp, except on the basis of an interpolated fifth bulb swelling. If such a fourth cusp were present in a solitary aortic trunk, the picture of the ideal four-cusped common trunk would be closely imitated. No such case has been reported, to my knowledge. However, when the unique case reported by Glas<sup>16</sup> is considered, it is easy to see that with modification in the direction of atresia of the sixth arch structures, identification might be difficult. The heart described by Glas showed a large aorta with four cusps, in the "rider" position, while the small, three-cusped pulmonary artery rose from a stenosed pulmonic conus.

Alterations in the relationship of semilunar cusps to adjacent structures have a bearing on the interpretation of anomalies. Normally, each arterial ostium has one primarily septal cusp, the left posterior of the pulmonary artery, and the right anterior of the aorta. The other two pulmonic cusps are in relation to the base of the nonseptal (conus) wall of the right ventricle. The medial end of the posterior aortic cusp is in contact with the membranous part of the interventricular septum, while its lateral part and a small adjoining part of the left aortic cusp lie above and are continuous with the anterior (aortic) cusp of the mitral valve. The rest of the left cusp is continuous with the base of the non-septal (conus) wall of the left ventricle.

*Muscular Structures of the Right Ventricle and the Right Aorta.*—On the basis of his phylogenetic studies, Spitzer<sup>8</sup> believed that in the mammal there is a rudimentary development of the second, right ventricular aorta of the reptile. He recognized as homologues of the muscle ridges of the inter-aortic septum of the reptilian heart the prominent muscle structures of the mammalian right ventricle. These structures were described by Tandler,<sup>4</sup> as the crista supraventricularis and the trabecula septomarginalis. Spitzer subdivided these muscle bands into the crista and trabecula proper, grouped as the crista aorticopulmonalis, and the anterior tricuspid ledge.

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16. Glas: Jahrb. f. Kinderh. 49:187, 1867.

The trabecula traverses the ventricle as a moderator band, from the lateral wall, near the acute margin and at a variable height above the apex, to the septum. Here the crista ascends as a smooth muscle band, to the base of the septal (left) pulmonic cusp. The ledge lies posterior and more or less fused with the crista. Its apical portion, lying behind the trabecula proper, is recognized from its intimate relation to the large lateral papillary muscle, which gives attachment to chordae from the anterior and posterior tricuspid leaflets. The septal part, commonly fused with the crista in the stem of the Y referred to in a foregoing paragraph, gives off several chordae for the medial tricuspid leaflet, and forms the base of the small septal papillary muscle of Lancisi, the medial attachment of the anterior tricuspid leaflet. Near the anterior margin of the membranous septum, this posterior part abruptly swings laterally and downward, in the direction of the large lateral papillary muscle. Near the base, this diverging muscle structure (arch of the crista of Tandler) separates the pulmonic ostium above from the anterior tricuspid leaflet, which is attached along its posterior wall. Thus, the almost complete muscular ring of the anterior tricuspid ledge demarcates the inflow (posterior) from the outflow (anterior) parts of the ventricle.

The importance of these structures in Spitzer's theory is that they identify the part of the ventricle belonging to the right aorta. The rudimentary conus of that vessel lies in a niche between these two structures at the base of the heart, directly opposite the conus of the left aorta. It is separated from that conus by the proximal bulb septum, from the inflow chamber by the ledge, and from the pulmonic outflow by the crista proper. Hence it ends blindly unless a septal defect is present. As has been seen, this region, the posterior part of the anterior septum of Rokitansky and the interaortic septum of Spitzer, is a common site of septal defects. When the septum is incomplete, and particularly when the aorta is shifted to the right, over the defect, important modifications are seen in the crista and ledge, and these changes may be the best clues one has as to the nature of the embryologic defect.

*Torsion and Detorsion.*—A factor that must be considered in the analysis of cardiac defects is the rôle of torsion, or of alterations in normal torsion, in relation to the picture produced. With lengthening and irregular widening of the primitive cardiac tube, a bending of the ventricular part develops. The winding of the ventricular loop produces a spiral rotation of the fixed ends of the tube. The normal direction of this winding is such as to produce a clockwise torsion of the arterial limb, and a counterclockwise twist of the venous end of the tube. Occasionally the direction may be reversed, giving the picture of mirror position dextrocardia, with or without heterotaxy. A torsion lag is a conspicuous feature of many cardiac malformations.

Spitzer stressed the rôle of detorsion in his phylogenetic theory of cardiac malformations. In his view, normal torsion, associated with local alterations due to the increased blood flow in the two circulations, is responsible for the definitive form of the mammalian heart. In other words, the development of a pulmonic circulation and normal torsion are essential for normal septum formation. At the arterial end the clockwise torsion finally reaches 180 degrees at the level of the semilunar cusps. In the embryonic stage it is documented, among other things, by the helical course of the swellings and septums of the trunk and bulb; in the definitive stage, by the clockwise winding of the pulmonary artery about the aorta. At the venous end, the counterclockwise torsion is most easily seen from the direction of the spirals of the interatrial septums, as seen from the right atrium.

Spitzer included the following features as landmarks of a lag in normal torsion: At the aortic ostium, the cusps and their identifying coronary ostia lag or are "displaced" in a counterclockwise direction. With extreme lagging there is a "transposition" of the two trunks, and one may see the "transposition" of the coronaries referred to heretofore. A defect in the interventricular septum is present, in the interaortic (conus) part. Spitzer interpreted this as due to opening up the conus of the right aorta, which may combine with the left ("common" or "rider" aorta) or may replace it (in "transposition of the great arterial trunks").

With increasing detorsion, the anterior tricuspid ledge grows smaller, and its divergence from the septum decreases. It finally loses its arch and comes to lie in the sagittal plane. As the ledge decreases and swings toward the septum, the attached anterior tricuspid leaflet naturally follows, until finally its medial part may stand in the same relationship to the semilunar cusps of the right or transposed aorta as the corresponding part of the anterior mitral cusp does to the normal aortic cusps. While the nonseptal part of the ledge becomes small the crista proper hypertrophies, and when aorta and pulmonary artery arise independently from the same (right) ventricle, it appears as a powerful muscle bundle ascending the septum and arching across the base to separate the two ostia. Thus it superficially resembles the arching part of the ledge. The more the pulmonic trunk is displaced to the left the larger the crista becomes, and the more it, too, approaches the sagittal plane. In so-called "crossed transposition," it and the septal part of the ledge may constitute a pseudoseptum, while only the rudiments of the true interventricular septum remain.

At the venous end, detorsion may be shown by disturbances in the relations of the two atria, and particularly by the presence of a widely patent foramen ovale with a poorly developed limbus (septum 2) and a shift of its valve (septum 1) to the left. With more extreme detorsion

there may be almost complete failure of septum formation, with persistence of a common atrioventricular ostium.

#### PARTIAL VS. TOTAL PERSISTENCE OF THE COMMON ARTERIAL TRUNK

The question of a qualifying adjective must be considered because cases have been reported as "total" or "complete" even in the presence of a well defined though abbreviated pulmonic trunk. Mönckeberg<sup>6</sup> and Feller<sup>17</sup> suggested that cases be considered as complete only when the spurs of the sixth arch have not fused, or when derivatives of the sixth arch are missing. This would include examples with an independent origin of the pulmonary arteries directly from the trunk, and those in which the lungs received blood only through collaterals. The cases in which there is very slight development of the septum could be qualified by such a term as "almost complete," to distinguish them from cases in which there are lesser septal defects, such as the two reported by Rokitansky<sup>18</sup> and the type of case reported and discussed by Hektoen.<sup>19</sup>

#### DIFFERENTIATION FROM SIMILAR ANOMALIES

The chief problem in establishing the identity of a common arterial trunk is due to the fact that superficially similar anomalies occur. These represent regression of one trunk after the aortic-pulmonary septum has formed and separated it from the other. Thus there are two types that must be distinguished, persistent solitary pulmonic trunk with aortic atresia, and persistent solitary aortic trunk with pulmonic atresia.

*Solitary Pulmonic Trunk.*—The anomalous heart possesses a single large arterial trunk, superficially combining the appearance of the aorta and the pulmonary artery. It should have three semilunar cusps and no coronary ostia. However, von Konstantinowitsch<sup>20</sup> reported an otherwise typical case in which one of the coronaries originated in a sinus of the pulmonic trunk. After giving off the pulmonary arteries at the usual level, the trunk goes over into the arch through an exceptionally wide ductus arteriosus. The impression conveyed is that of a continuous trunk, although in a number of reported cases the large branches of the arch appear to be crowded together or irregularly arranged. Aside from the absence of coronary ostia, the identifying feature is a small artery, the remnant of the aorta. This originates from the arch or from the root of one of its branches and follows the approximate course of the

17. Feller, A.: Virchows Arch. f. path. Anat. **279**:869, 1931.

18. von Rokitansky, C. F.: Die Defekte der Scheidewände des Herzens, Vienna, W. Braummüller, 1875; cited by Mönckeberg,<sup>6</sup> Abbott<sup>2</sup> and Keith.<sup>25</sup>

19. Hektoen, Ludvig: Am. J. M. Sc. **121**:163, 1901.

20. von Konstantinowitsch, W.: Prag. med. Wchnschr. **31**:657, 1906.

aorta with reference to the pulmonic trunk. At the base of the heart it gives off the coronary arteries and is inserted blindly above a left ventricle, which is often rudimentary.

Since Mayer's<sup>21</sup> report many examples of this anomaly have been doubly misinterpreted. The solitary pulmonic trunk has been regarded as a persistent common trunk. The aortic remnant, in which, obviously, the direction of blood flow is reversed, has been interpreted as a coronary artery, with an abnormally "high" origin. Since Rauchfuss,<sup>22</sup> Vierordt,<sup>23</sup> Herxheimer,<sup>1</sup> Abbott<sup>2</sup> and Mönckeberg<sup>6</sup> have emphasized the incorrectness of this interpretation, there is little excuse for failing to recognize this defect. A typical case has recently been reported by Shapiro.<sup>3</sup>

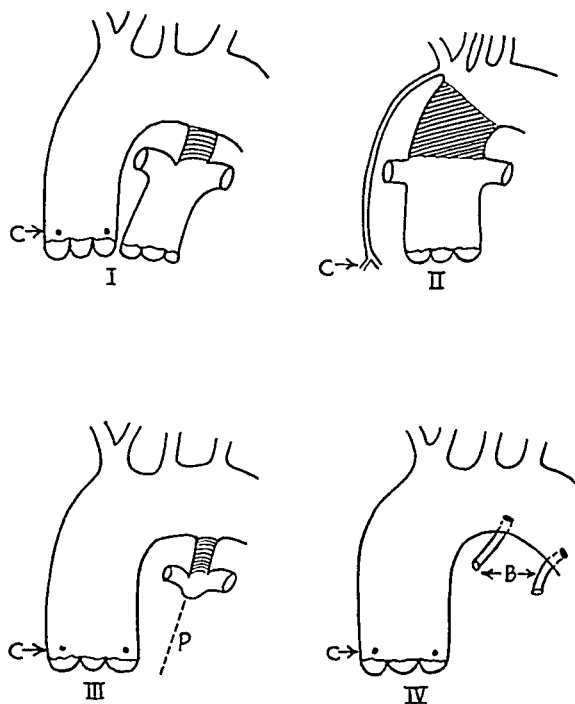


Fig. 1.—Diagrams of types of solitary arterial trunk: *I*. Normal separation of the aorta and pulmonary artery in a normally developing heart. *II*. Solitary pulmonic trunk, with an atretic aorta. The aortic remnant gives off the coronary arteries (*C*). *III*. Solitary aortic trunk, with an atretic pulmonic trunk. The pulmonary arteries receive blood through a patent ductus. The remnant of the pulmonic trunk may or may not be recognizable (*P*.) *IV*. Solitary aortic trunk, with bronchial arteries, and no recognizable sixth arch derivatives. In all four diagrams the ductus arteriosus is shaded. *C* indicates coronary ostia; *B*, bronchial arteries.

21. Mayer: *J. d. Chir. u. Augenh.* **10**:44, 1827.

22. Rauchfuss, C.: *St. Petersburg. med. Ztschr.* **6**:370, 1864.

23. Vierordt, Hermann: *Die angeborenen Herzkrankheiten*, in Nothnagel: *Spezielle Pathologie und Therapie*, Vienna, Hölder, 1898, vol. 15, pt. 1, sect. 2.

*Solitary Aortic Trunk.*—In this anomaly, too, there is a single arterial trunk, which, with few exceptions, resembles the normal aorta. Thus one would expect three semilunar cusps and two coronary ostia. However, in two otherwise typical examples recently reported by Kugel<sup>24</sup> but one coronary stem was present. Also it is theoretically possible, as shown by Glas'<sup>16</sup> case, for four cusps to be present.

According to the degree of regression of the derivatives of the sixth arch, the pulmonary blood supply may vary. A well developed ductus arteriosus may persist, giving off the right and left pulmonary arteries. The atretic remnant of the pulmonic trunk may be present as a fibrous

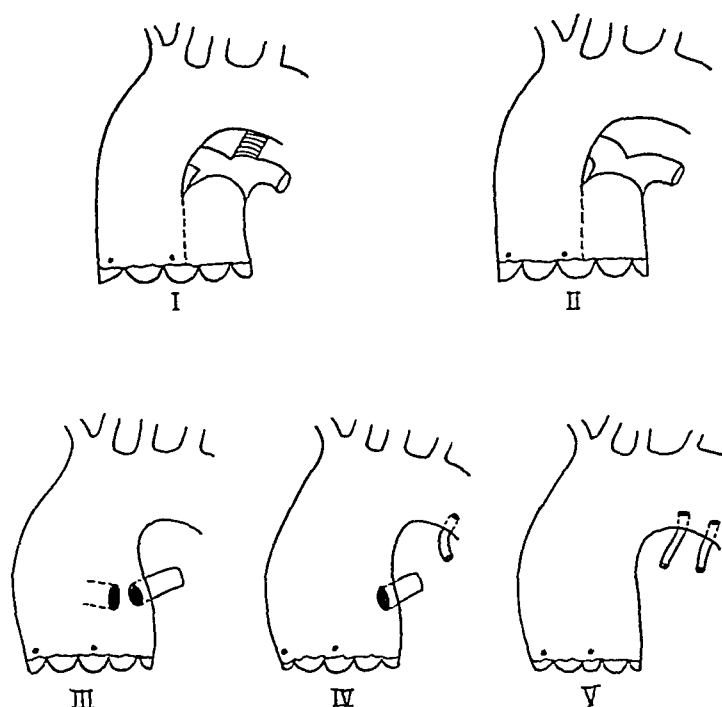


Fig. 2.—Diagrams of types of common arterial trunk: *I*. Partial common trunk with ductus arteriosus. *II*. Partial common trunk without ductus. *III*. Complete common trunk, with independent origin of two pulmonary arteries. *IV*. Same, transition form with one pulmonary and one bronchial artery. *V*. Same, with no sixth arch derivatives and only bronchial arteries.

cord, as in the case illustrated by Keith.<sup>25</sup> With more marked regression, the sixth arch vessels may be small; one of the pulmonary arteries may be missing, or there may be total disappearance of both sixth arches. Keith found this to be frequent in his cases. With diminished blood flow through the pulmonic channels, the collateral vessels, particularly the bronchial arteries, widen, and with atresia, the sole pulmonic arterial

24. Kugel, M. A.: *Am. Heart J.* **7**:262, 1931.

25. Keith, A.: *Lancet* **2**:359, 433 and 519, 1909.

blood supply goes through these vessels. A similar condition is seen in pulmonary atresia without cardiac deformity (Christeller,<sup>26</sup> Müller<sup>27</sup>).

Pulmonary and bronchial arteries may be distinguished by their origin and by their manner of entrance into the lungs. The former originate from the trunk, usually near the pericardial attachment, and enter the hilus close to and anterior to the main bronchus. The latter are often multiple and are variable in size. They originate irregularly from the arch and descending aorta and enter the hilus irregularly, often above or behind the bronchus, and frequently branch before entrance.

*Identification of the Anomaly.*—The typical example of solitary pulmonic trunk should be easily recognized. However, the aortic character contributed by a misplaced coronary, in a case like that of von Konstantinowitsch,<sup>20</sup> might cause confusion unless the aortic remnant is clearly recognizable.

The real difficulty is to distinguish the "complete" common trunk from solitary aortic trunk, when remnants of sixth arch structures are not found. Unless fibrous remnants of these structures are identified, a differentiation of the anomaly involving aplasia or very early regression of the sixth arch structures from that involving atresia and disappearance of the separated pulmonic trunk cannot be made on the character of the arterial trunk alone. The differentiation is further complicated by the fact that, in this group, as in most cases of common trunk, the aorta occupies the "rider" position. Thus the defect is a combination of various degrees of "transposition" with atresia of the pulmonic element. Another factor that must be considered is that in the hearts of adults, in this class, remnants of sixth arch structures that might have been recognizable in infancy may no longer be distinguished.

It remains to be seen whether a careful study of septal defects and of the muscular structures of the right ventricle will assist in the differentiation. On theoretical grounds these should give valuable information. If one found a well developed crista arching the vault of the ventricle, anterior to the solitary arterial ostium, this would suggest atresia of a "transposed" pulmonic trunk. The probability would be increased if the arch demarcated a small diverticulum, representing the pulmonic conus. With a common trunk, on the other hand, the crista should end at the base of the septum, beneath the anterior septal cusp.

#### REPORT OF A CASE

*Clinical History.*—A boy, born at full term at the Women and Children's Hospital, Chicago, on June 1, 1931, showed signs of congenital cardiac lesion at birth and atresia of the rectum. An operation was performed for production of an

26. Christeller, Erwin: *Virchows Arch. f. path. Anat.* **223**:40, 1916.

27. Müller, Leo: *Ztschr. f. Kreislaufforsch.* **19**:561, 1927.



artificial anus. The first bowel movement was one of meconium; it was followed by repeated stools of bloody mucus. There was persistent vomiting, with regurgitation of blood-stained fluid. The infant died on June 6.

*Postmortem Examination.*—Autopsy was performed by Dr. M. A. Southwick on the day of the infant's death. The findings were: a surgically created anus; a pocket-like invagination at the upper end of the natal fold; a palpable defect in the sacral arches (spina bifida occulta); a normal situs of the heart and other viscera; general cyanosis and venocapillary congestion; an abnormal arrangement of the mesenteries; a mobile cecum; bands between the gallbladder and the duodenum; multiple hemorrhages in the mucosa of the entire gastro-intestinal tract, with superficial ulceration in the colon; subcapsular hemorrhages in the liver, and pulmonary hyperemia and edema.

*Histologic Examination.*—The chief observations were capillary engorgement, marked passive hyperemia of the liver, with necrosis of the central zones, and pulmonary hyperemia and edema.

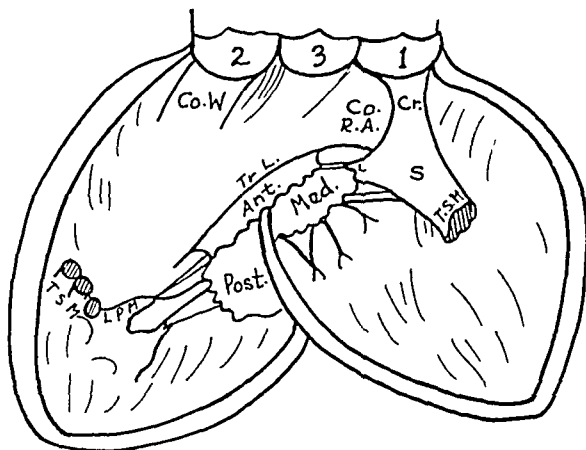


Fig. 3.—Diagram of a normal right ventricle of a child, aged 5 years. The numerals 1, 2 and 3 indicate cusps of the pulmonary artery; *Cr.*, the crista supra-ventricularis; *Tr.L.*, the anterior tricuspid ledge; *L.*, the papillary muscle of Lancisi; *L.P.M.*, the lateral papillary muscle; *S*, the fused septal part of the ledge and crista; *T.S.M.*, the trabecula septomarginalis; *Ant.* and *Med. Post.*, the tricuspid leaflets; *Co.W.*, the anterior wall of the pulmonic conus; *Co.R.A.*, the conus of the right aorta.

*Description of Heart.*—The length from the apex to the summit of the atria was 5.25 cm.; from the base of the arterial trunk to the apex, anteriorly, 4 cm.; from the atrioventricular groove to the apex along the acute margin, 4 cm., and along the obtuse margin, 2.75 cm. The maximum transverse diameter was 3.5 cm.; the anteroposterior diameter, 3 cm.

The heart was enlarged and rounded, and the apex was directed to the left. The anterior interventricular furrow appeared to be farther to the left than normal, while a proportionally large part of the presenting surface and the rounded apex was made up of the right ventricle. The two auricles stood in normal relation to the four chambers, although the left seemed small and was displaced a little posteriorly. There was no special prominence of the infundibular region of the right ventricle. In fact, the entire upper part of the anterior and lateral wall of this chamber appeared rounded and prominent. Only one large arterial trunk was

present, occupying most of the base of the heart. On its outer surface there was no indication of a subdivision. It bulged to the right immediately above its origin. From its posterior part, on the left, right and left branches were given off. The right passed behind the main trunk to enter the hilus of the right lung anterior to the main bronchus. The left passed to the left lung and entered similarly. From the angle between the two branches, apparently the pulmonary arteries, a third vessel emerged, and subsequently joined the main trunk. This vessel, 6 mm. long and appearing somewhat flattened, was a ductus arteriosus. The right half of the trunk continued into a normally constituted aortic arch, which gave off the innominate, left carotid and left subclavian arteries. The point of junction with the ductus arteriosus was the concavity of the arch, opposite to and just distal to the left subclavian. The subsequent course of the vessel was that of the normal descending aorta.

The ostium of the solitary trunk was equipped with four semilunar cusps. These were thick and fleshy, with irregular outer surfaces, covered with smooth endothelium. Instead of having a distinct nodulus, each cusp terminated in a point. The cusps were arranged so that two were anterior and two posterior. The right

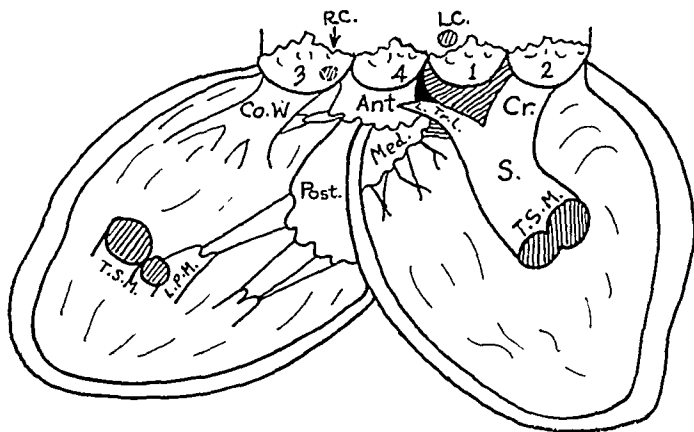


Fig. 4.—Diagram of the right ventricle in the case reported in this paper. The numerals 1 and 3 indicate undivided cusps; 4, the "posterior" (aortic) cusp, and 2, the "anterior" (pulmonic) cusp of the common trunk; L. C., the left coronary ostium; R. C., the right coronary ostium. The septal defect is represented by a shaded region beneath cusp 1, bordered posteriorly by the membranous part of the septum (black). Other structures are labeled as in figure 3.

anterior cusp was slightly in advance of the left. Numbering the cusps in a clockwise direction, cusp 1 (above which the left coronary arose) was left posterior; cusp 2, left anterior; cusp 3 (back of which the right coronary had its origin), right anterior, and cusp 4, right posterior. They were almost equal in size, cusps 1 and 4 measuring 7 mm. between the commissures; cusps 2 and 3, 6 mm. Reasoning from the position of the coronary arteries, cusp 2 (left anterior) represented the anterior pulmonary cusp; cusp 4, the posterior (noncoronary) aortic cusp, and cusps 1 and 3, the undivided "lateral" swellings.

The coronary ostia stood in their normal relationships to each other and to the cusps. Thus the left coronary rose just above the margin of cusp 1 (left posterior), and shifted slightly toward the commissure with cusp 4 (right posterior). The right coronary originated deep in the sinus of cusp 3 (right anterior) and close to its junction with cusp 4. The main stems of the arteries seemed long, particularly

that of the left, the origin of which lay farther back than usual. The left circumflex branch was short and soon terminated on the anterolateral surface of the left ventricle as an oblique descending branch. The left anterior descending branch was normal. The right coronary followed its usual course, gave off the posterior descending branch, and continued on to the posterior wall of the left ventricle.

Distally, the left half of the trunk was separated from the right by an archlike demarcation, obliquely placed, and slanting from the right posteriorly to the left anteriorly. The supports of the arch were seen as two very low ridges, extending from the vault to the arterial ostium. The posterior ridge met the commissure between cusps 1 and 4; the anterior, the opposite commissure between cusps 2 and 3. As viewed from above, these ridges described a clockwise spiral, but with less of a twist than would result if they reached the midpoints of cusps 1 and 3, the normal points of transection of the cusps and ostium. These ridges divided the ostium into two equal halves, 1.3 cm. each in circumference. Just above the ostium, in the bulging region, the right part measured 1.8 cm.; the left, 1.3 cm. As was indicated from the external examination, the right half continued into a normally constituted aortic arch and a descending aorta, the circumference of which at the level of the ductus arteriosus was 1.3 cm., while distally it increased to 1.5 cm.

The vault of the arch, 1.2 cm. above the bases of the cusps, cut off the shallow end of the left half of the trunk, from which the three sixth arch vessels originated. The arch was seen to be the margin of a low crescentic infolding. Below the level of the vault the mouth of the right pulmonary artery, 4 mm. in diameter, was seen. Concealed beneath the margin of the fold were the openings of the ductus arteriosus and the left pulmonary artery, which appeared to rise from the first part of the ductus. It was the same size as the right, while the ductus had a circumference of 6 mm. The inner surface of the latter was a little wrinkled. The relationships here certainly indicated the formation of an abbreviated pulmonic trunk, from which the normal derivatives of the sixth arch arose. However, the forerunners of the rudimentary septum, which indicated a division of the common trunk, failed to meet the midpoints of cusps 1 and 3, by a detorsion estimated at 45 degrees. Because of this lag, the left coronary ostium lay in the region marked off as belonging to the pulmonary artery. This is of interest because of the fact that one coronary, usually the left, has been reported as originating from the pulmonary artery. It would seem, however, that when this displacement is the only anomaly, it can be accounted for more easily by a displacement of the coronary anlage, than by a shift of the septum.

The right atrium received the two venae cavae and the coronary sinus in normal fashion. The eustachian valve was distinguishable as a fleshy fold, and a small thebesian valve was present at the mouth of the sinus. The left atrium received a common venous trunk, 4 mm. long, from the left lung, while the two right pulmonic veins entered independently. The primitive interatrial foramen was closed, but there was a wide foramen ovale (secundum). Its limbus was low, but was well demarcated, especially inferiorly. Its opening was partially guarded by a low crescentic fold (septum primum). This started on the upper anterior wall of the left atrium. At first it ran medially, inferior to and marking off the horn of the atrium that received the right pulmonic veins. It then curved backward and downward, gradually approaching the limbus, and ended on the anterior inferior wall of the atrium. The greatest width of the crescent was 4 mm., and it was separated from the limbus by a distance of 5 mm. above, decreasing to 0.5 mm.

The right ventricle was capacious, and its thick wall measured from 3 to 4 mm. Its muscle columns were conspicuous, and there were several features worthy of note in their arrangement. On the anterior wall of the conus beneath cusp 3

(right anterior), a heavy smooth muscle descended and soon broke up into trabeculae on the anterior wall. On the septal wall was a heavy Y-shaped muscle structure with a smooth surface. The vertical limb was short and broad, and slanted from anterior below to posterior above. Beneath a defect in the septum, it branched. The anterior limb formed the border of the defect anteriorly and ascended to the base of the septal semilunar cusp 2 (left anterior). The other formed the base of the defect posteriorly and, tapering gradually, skirted a small membranous part of the septum, and was attached to the anterior surface of the medial half of the anterior tricuspid leaflet. From its posterior margin, below its pointed end, several short chordae tendinae went to the medial tricuspid leaflet. From the base of the Y a very heavy muscle crossed the ventricular cavity as a short, thick moderator band, which appeared to be made up of two parts. The large anterior part was attached directly to the wall, while the smaller was attached to the base of a large lateral papillary muscle. Comparison with the normal showed that the large moderator band undoubtedly was the trabecula septomarginalis. Its small posterior

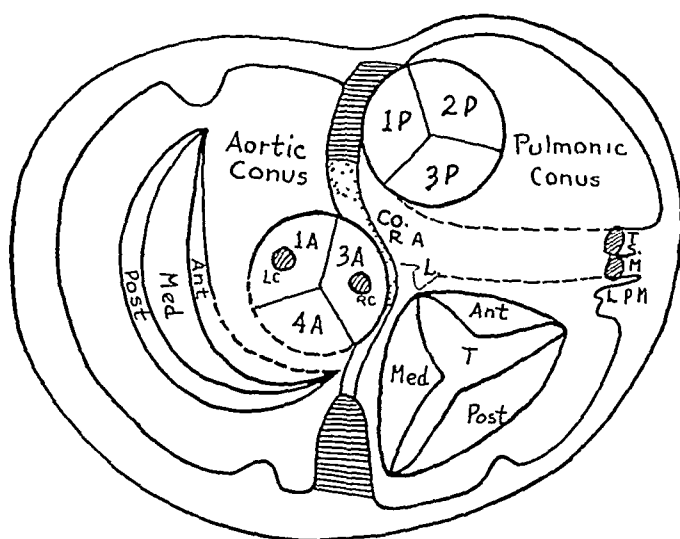


Fig. 5.—Diagram of a normal heart, showing the relations of the valve orifices to adjacent structures. *A* indicates the aortic cusps; *P*, the pulmonic cusps. The shaded regions of the interventricular septum indicate the anterior and posterior muscular portions; the stippled region, the interaortic (muscular) septum; the unmarked region, the membranous septum. The dotted lines at the bases of the aortic and anterior mitral cusps show the region of fusion.

part, in relation with the papillary muscle, represented the lateral apical part of the anterior tricuspid ledge. Both the ledge and the crista supraventricularis were fused where they ascended the septum, in the stem of the Y. Beneath the defect, the crista forked anteriorly and ascended to the base of cusp 2. This is contrary to its behavior when the arterial trunks are transposed; then the hypertrophied crista traverses the base like an arch, between the two ostia. The ledge turned posteriorly and soon ended in a point, which seemed to represent an elongated muscle of Lancisi. In other words, the ledge had lost its normal divergent arch.

Correlated with the hypoplasia of the tricuspid ledge there were striking changes in the relations of the anterior tricuspid leaflet. It appeared as a short cusp, with its medial end shifted toward the sagittal plane, and throughout most of its length it was applied directly to the base of semilunar cusp 4 (right posterior); i. e., it

stood in the same relation to the noncoronary aortic cusp as does the anterior mitral leaflet in the normal heart. Associated with its decrease in size, loss of obliquity and shift of plane was its detachment from the large lateral papillary muscle. Its lateral attachments were a few short chordae, lateral to the muscle bundle beneath cusp 2 (right anterior), originating close to the base of the heart.

The relations of the posterior and medial tricuspid cusps were not greatly altered, though the medial was small and the posterior relatively large. There were two well defined groups of papillary muscles. Attached to the posterior cusp were the chordae from the large lateral muscle described, and others from a second smaller group, lying behind it. The chordae of the medial cusp rose from a group of small papillary muscles situated high on the posterior septum, and from the septal part of the tricuspid ledge, as described.

The left ventricle had a relatively small cavity and a wall from 4 to 6 mm. thick. The muscle columns were small and flat, and the vertically directed aortic conus sloped toward the septal defect. Unlike the normal, none of the conus lay

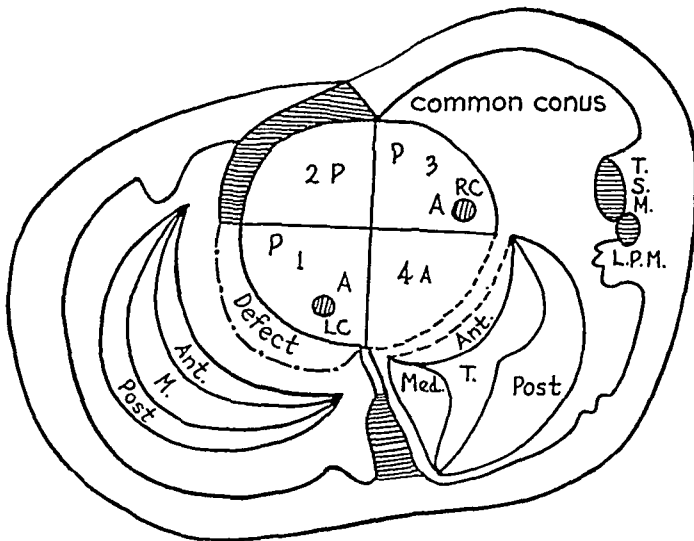


Fig. 6.—Diagram of the heart in the case reported in this paper, showing the relations of the orifice of the common trunk to adjacent structures. *A* indicates the aortic and *P* the pulmonic elements of the cusps. A defect is represented in the interaortic part of the interventricular septum, beneath cusp 1. The dotted lines show fusion of cusp 4 with the anterior tricuspid leaflet.

anterior to the mitral area, but it appeared both flattened and shortened antero-posteriorly, so that the anterior mitral cusp hung like a curtain against the defect. The basal attachment of this cusp was entirely muscular; i. e., it was not fused with the base of the aortic cusps as in the normal heart.

The interventricular septum showed a helical twist. The base measured 1.5 cm. in a straight line, and 2.2 cm. along its curving margin. It sloped from right above posteriorly to left below anteriorly. At the base, the greatest convexity was anterior and directed to the left. At the apex, the convexity was toward the right, and the tip of the left ventricle, lying in advance of the right, appeared as if holloed out of the septum. The greatest breadth of the septum, 1 cm. below the base, was 3 cm., and its average thickness was 5 mm.

The septal defect was pyramidal in shape. Its base, along the lower margin of semilunar cusp 1 (left posterior), measured 7 mm., and its height at the

branching of crista and ledge was 5 mm. As stated, its lower margins were these two muscle structures, which were fused smoothly with the rest of the anterior septum. At its posterior angle it was in relation with a small part of the base of cusp 4 (right posterior) at its junction with the anterior tricuspid leaflet. And just behind this, it bordered the small membranous part of the septum, interposed between it and the posterior muscular septum. Hence the base of the septum, from front to back, was made up of an anterior muscular part, measuring 8 mm., the defect, 7 mm., and a posterior septum, 7 mm., the anterior membranous part of which measured about 2.5 mm. It should be noted that the small membranous septum was normally placed beneath the attachment of the medial tricuspid leaflet. A needle passed from the point where it joined the defect emerged in the right atrium, below and anterior to the mouth of the coronary sinus. These relationships indicate that the defect was in the posterior part of the anterior septum of Rokitsansky, the interaortic septum of Spitzer.

The basal relations of the semilunar cusps were as follows. The base of cusp 1 (left posterior) bridged the septal defect, as described. A small part of the adjacent border of cusp 4 (right posterior) bordered the defect, in the region of the membranous margin, beyond which the base of this cusp was closely applied to the base of the anterior tricuspid leaflet. Cusp 3 (right anterior) lay above the base of the anterior (conus) wall of the right ventricle. Cusp 2 (left anterior) was applied to the base of the anterior part of the interventricular septum. Thus two cusps, instead of one, were in relation to the septum, while only cusp 1 had any appreciable relation to the defect. Almost three fourths of the entire circumference belonged exclusively to the right ventricle, and only half of the cusp that bridged the defect was developmentally an aortic cusp. Taking the positions of cusps 2 and 4 as indexes, the arrangement indicated a detorsion defect, probably of slightly more than 45 degrees.

Summary: Summarized, the observations on the heart showed a normal situs; a four-chambered heart; partial (almost complete) persistence of the common arterial trunk; a rudimentary aortic-pulmonary septum, delimiting an abbreviated pulmonic trunk, which gave off in normal fashion right and left pulmonary arteries and a ductus arteriosus; four semilunar cusps, with normal relations of cusps and coronary ostia, position of cusps and ostia indicating a lag in a counterclockwise direction of about 45 degrees from normal; polypoid overgrowth of the semilunar cusps; halving of the trunk ostium by forerunners of the aortic-pulmonary septum, their positions indicating a shift of 45 degrees in a counterclockwise direction from the expected line of descent, i. e., total detorsion of the septum of about 90 degrees; location of the left coronary ostium in the pulmonic part of the trunk; general course of the coronaries normal; a defect in the base of the interventricular septum, in the posterior part of the anterior septum; "rider" position of the common trunk, with major origin from the right ventricle; hypertrophy of both ventricles, particularly the right; opening up of the right aortic conus, with decrease in the size of the left aortic conus and loss of its anterior (premitral) part; dissociation of the anterior mitral cusp from the base of the arterial trunk, with attachment of the medial part of the anterior tricuspid leaflet to the base of the trunk; shift of the position of the latter cusp toward the sagittal plane, with high attachment of its lateral end to the basal part of the ventricular wall; fusion of the medial end of this cusp with the elongated papillary muscle of Lancisi; absence of the basal arch of the anterior tricuspid ledge; hypertrophy of the trabecula septomarginalis and the septal parts of the crista supraventricularis and the anterior tricuspid ledge; a remnant of the membranous part of the interventricular septum in normal position at the posterior margin of the defect and in relation to the medial end of the

anterior tricuspid leaflet; hypertrophy of the right atrium; small left atrium, its auricle displaced backward; wide patency of the foramen ovale (secundum); hypoplasia of both the limbus and the valve, with displacement of the valve (septum primum) to the left; common right pulmonic venous trunk.

#### REVIEW OF THE LITERATURE

Part of the difficulty in an attempt to survey the literature on the common arterial trunk arises from confusion of nomenclature. In some reports the term "persistent common arterial trunk" appears to be used in the sense that the trunk is common to both ventricles. In the older literature many cases are reported as "persistent arterial trunk" without reference to the developmental features. In other reports the data are incomplete, particularly with reference to such important identifying features as the coronary blood supply and the arrangement of the semilunar cusps. In others there is no description of the arterial supply of the lungs, a knowledge of which is essential for diagnosis. In very few is there any mention or description of the muscle bundles of the right ventricle. Another fact that must not be forgotten is that true cases of persistent common trunk may be hidden under the caption of pulmonic atresia.

Keith<sup>25</sup> believed that this anomaly was very rare, and stated that he had never encountered an example, personally. In his discussion of the condition, he presented a picture of one of Rokitansky's two cases. This shows a partial defect, with the lower end of the septal margin at the level of the interventricular defect. Herxheimer<sup>1</sup> listed forty-three cases, but many of them have been positively identified as examples of solitary aortic or pulmonic trunk. His description of a "typical" case, in a specimen from the Heidelberger Institute, permits identification as an example of solitary aortic trunk. The "pulmonary" artery from the arch, giving off right and left pulmonic branches, undoubtedly represents a ductus arteriosus. Abbott,<sup>2</sup> as has been stated, found twenty-three cases, fourteen of which she analyzed as instances of the "complete" defect. The specimen from the McGill Museum, described by her, had three semilunar cusps and two coronary ostia. However, she stated that the blood supply to the lungs was unknown, which makes positive identification impossible. Mönckeberg<sup>6</sup> discussed the features of his anomaly and the difficulties of identification. He used as an example Wirth's<sup>12</sup> specimen, the original report on which is difficult of access. This example fulfils many of the criteria.

A number of excellent critical reviews have appeared in recent years. Those of Hülse,<sup>14</sup> Siegmund<sup>28</sup> and Feller<sup>17</sup> are especially valuable. Hülse quoted the analyses of Pietzch<sup>13</sup> and Wirth,<sup>12</sup> who admitted only

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28. Siegmund, H.: *Ztschr. f. Kreislaufforsch.* **20**:65, 1928.

the cases of Buchanan<sup>29</sup> and Preiz<sup>30</sup> and Wirth's own case as true instances of total persistence of the common trunk. Hülse added one case and included two cases reported by Wenner<sup>31</sup> and, as probable, one reported by Wright and Drake.<sup>32</sup> Siegmund added three cases, two of his own and one reported by Klemke.<sup>33</sup> Feller added Zimmermann's<sup>34</sup> case and four of his own, three of them cases of "partial" defect. To these should be added the case of Dickson and Fraser,<sup>35</sup> possibly those of Finley<sup>36</sup> and Grant,<sup>37</sup> and certainly that of Santa Cruz<sup>38</sup> and the case reported in this paper. The completed list includes but twenty cases that have been regarded by recent reviewers as probable instances of the anomaly under consideration. It does not include cases with such minor defects as those seen in the group discussed by Hektoen,<sup>19</sup> nor those with partial, but almost complete, septums, such as Rokitansky's<sup>18</sup> two cases.

*Analysis of Reported Cases: Group A. Persistent Common Arterial Trunk with Four Semilunar Cusps.*—This group includes four cases of partial (almost complete), and one of complete, common trunk. They have certain features in common. All were found in infants, the oldest aged 2½ months, who had shown cyanosis and other signs of congenital heart disease. In each case, the four semilunar cusps showed the changes sometimes referred to as "fetal endocarditis"; that is, they were variously described as firm and thick, with warty or polypoid surfaces. Histologic studies showed only noninflammatory proliferative changes. Feller<sup>17</sup> called attention to the fact that they resembled normal fetal cusps. In all of these cases, the heart had four chambers, with a defect in the interventricular septum, anterior to at least a part of the membranous portion of the septum. (Santa Cruz made no reference to this feature). In other words, the defect was in the posterior part of the anterior septum of Rokitansky, the interaortic septum of Spitzer. In each case, the common trunk rode the defect, in most instances shifted to the right so that the major part of its ostium was related to the right ventricle. The part related to the defect and thus to the left ventricle was usually in the posterior left quadrant. There was a correlated hypertrophy of the heart, predominantly of the right chambers. The

29. Buchanan, G.: Tr. Path. Soc., London, **15**:89, 1864.

30. Preisz, Hugo: Beitr. z. path. Anat. u. z. allg. Path. **7**:247, 1890.

31. Wenner, Otto: Virchows Arch. f. path. Anat. **196**:127, 1909.

32. Wright, J. H., and Drake, A. K.: Tr. A. Am. Physicians **18**:272, 1903.

33. Klemke, W.: Centralbl. f. allg. Path. u. path. Anat. **36**:307, 1925.

34. Zimmermann, H. M.: Am. J. Path. **3**:617, 1927.

35. Dickson, W. E. C., and Fraser, J.: J. Anat. & Physiol **48**:210, 1910.

36. Finley, K. H.: Am. J. Path. **6**:317, 1930.

37. Grant, H. H.: Am. J. M. Sc. **86**:149, 1883.

38. Santa Cruz, J. Z.: J. Philippine Islands M. A. **5**:295, 1925.



foramen ovale was open, except in the oldest infant. With the exception of Preisz' case, the arch was normally formed, but for the variant of a right aortic arch in one of Feller's cases. A histologic study of the myocardium by Feller showed only hypertrophy of myocardial fibers and a little interstitial fibrosis. In all cases, the situs was normal, and the lungs were normally formed. The special features of each case are as follows:

Preisz'<sup>30</sup> case was that of a girl 9 hours old. The observations were: four semilunar cusps, two posterior and two anterior; a coronary artery with distribution to the right ventricle from the left anterior sinus; a coronary for the left side of the heart from above the commissure between the posterior cusps; a bulging trunk, its division into two parts indicated by a shallow groove; the right part, small, flattened, giving off two carotid arteries and the right subclavian artery; from the posterior wall of the left part, two arteries to the right lung, at the level of the arch branches; a little further, on the same side, an artery to the left lung, and opposite, the left subclavian; further course like that of the normal descending aorta; a foramen ovale with a perforated membrane displaced to the left and widely patent; a tricuspid valve "normally formed," but with the anterior cusp in relation to the anterior margin of the septum membranaceum; a powerful muscle bundle springing from the anterior wall of the right ventricle (trabecula septomarginalis?); an interventricular foramen below the middle of the left posterior cusp, therefore with less than a quarter of the circumference related to the left ventricle.

There are certain puzzling features to this case. The groove probably represented the anlage of the aortic-pulmonary septum. It is hard to explain why the right, obviously aortic, half seemed to end with the three arch branches, while the trunk appeared to continue from the left, pulmonic half. The most rational explanation would be that the sixth arch structures at least partially differentiated, but only a short wide ductus persisted, taking the place of an atretic distal aortic arch. The relation of the left subclavian to the ductus suggests this, as, according to Abbott,<sup>2</sup> the apparent origin of this vessel from the distal end of the ductus is not an infrequent anomaly. Consideration of Feller's case 3 makes this interpretation probable. The arteries to the lungs were probably, from their origin, bronchial arteries, though there is no reference to their mode of entrance into the lungs. Another puzzling feature is the relationship of the coronary arteries. It seems probable that the one arising over the commissure belonged to the right posterior cusp. The exact origin of the one from the left anterior sinus is not given, but the shift of the posterior would seem to indicate that the left posterior cusp represented the noncoronary cusp. Feller,<sup>17</sup> however, concluded that it was much more likely that the posterior (left) coronary was displaced to the pulmonic field (as in my case), and that the right anterior cusp represented the noncoronary aortic cusp, which would indicate a severe detorsion defect of from 135 to 180 degrees. The alternative explanation would require a contratorsion, of which there was no evidence in the position of the viscera. The evidence, then, is in favor of

a diagnosis of total persistence of the common trunk, with a persistent ductus arteriosus compensating for an atresia of the distal aortic arch, and with a severe detorsion defect, but without transposition of the coronaries (evidenced by the long course of the left coronary, about the left side of the trunk).

Santa Cruz's<sup>38</sup> case was that of a boy 63 days old. He found four semilunar cusps, anterior and posterior, and right and left; a well defined aortic-pulmonary septum descending to 1 cm. above the cusps, with a forerunner indicating a bisection of the right cusp; a location of the pulmonic part of the trunk anteriorly and to the left, which gave off from its posterior wall the two pulmonary arteries; an absence of the ductus arteriosus; a normal arch and descending aorta.

This description lacks certain important details, as there is no mention of the coronary arteries, nor of the relation of the septal defect to the cusps and the membranous septum. However, it is sufficiently detailed to identify this as an example of a partial persistence of the common trunk. The position of the forerunner, bisecting the right cusp, gives a clue to the degree of detorsion, which must be slight, probably less than 20 degrees.

Feller's<sup>17</sup> case 1 was that of a prematurely born girl at 8 months who lived two days. His observations were: four semilunar cusps, two posterior and two anterior; a left coronary artery from the sinus of the left posterior cusps and a right coronary from the right anterior cusp, both shifted toward the commissures with the right posterior (i. e., noncoronary) cusp; a distinct aortic-pulmonary septum marking off a short pulmonic trunk, which gave off two normal pulmonary arteries; absence of the ductus arteriosus; definite forerunners of the septum, the anterior (left) stopping high and the posterior (right) stopping above the middle of the left posterior cusp; absence of abnormalities in the arch and descending aorta, except for a right instead of a left course; a slightly developed "crista supraventricularis" (arch of the tricuspid ledge?).

The description given is unquestionably that of a partial common trunk. The cusps are identified from the "normal" origin of the coronaries, and their position indicates a detorsion defect of a little less than 45 degrees. The long forerunner of the aortic-pulmonary septum lies in the expected position for bisecting the left posterior cusp; i. e., its detorsion coincides with that of the trunk.

Feller's<sup>17</sup> case 4 was that of a girl 2½ months old. The observations were: four semilunar cusps, two posterior and two anterior; a left coronary artery from the sinus of the left posterior cusp, almost above the commissure with the right posterior cusp; a right coronary artery from the posterior end of the right anterior cusp; a septal defect beneath both posterior cusps, so that nearly half of the circumference was related to the left ventricle; a distinct aortic-pulmonary septum, with forerunners high above the ostium; a short pulmonic trunk, giving off two normal pulmonary arteries and a still patent ductus arteriosus; a well formed "crista"; a small muscle band from the septum (muscle of Lancisi?) forming part of the attachment of the anterior tricuspid leaflet; a closed foramen ovale; a normal arch and descending aorta.

Here, too, the description is that of a partial common arterial trunk, with a detorsion defect similar to that in the preceding case, of probably less than 45 degrees.

The case reported in this paper was that of a boy 6 days old. For details, see the earlier summary. In general features, this case resembles Feller's cases 1 and 4, but with slightly greater detorsion—more than 45 degrees. As in Feller's case 4 there is a ductus arteriosus. It shows also a further lag of the aortic-pulmonary septum of 45 degrees with a consequent displacement of the left coronary to the pulmonic field. It is impossible to compare other features; but in my case there is additional evidence of greater detorsion at the venous end, in the more primitive state of the atrial septums.

*Group B. Persistent Common Arterial Trunk with Three Semilunar Cusps and a Rudimentary Fourth Cusp.*—The general characteristics of the first two of the three cases in this group are very similar to those of group A.

Buchanan's<sup>29</sup> case was that of a girl 6½ months old. The observations were: three cusps, a left anterior and a left posterior and a large right, with a ridge indicating a subdivision of this cusp into anterior and posterior halves, so that it "looked much like two imperfect valves joined together"; same "knotted" consistency of cusps as in group A;; two large pulmonary arteries given off from the common trunk before it left the pericardial sac from "above the right coronary"; absence of a ductus arteriosus; a normal arch and descending aorta, except for the right course; a trunk from both ventricles; a wide open foramen ovale. The exact relation of the ventricular septal defect is not stated. No description of the coronary arteries is given.

The data do not permit the identification of the cusps and the degree of detorsion. However, the independent origin of the pulmonary arteries from the first part of the trunk makes it probable that this is an example of complete persistence of the common trunk.

Feller's<sup>17</sup> case 2 was that of an infant several months old. The observations were: three semilunar cusps, anterior, posterior left and posterior right; thick, slightly irregular cusp margins; partial fusion of the two posterior cusps at their commissure; indicated subdivision of the right posterior cusp; a well defined aortic-pulmonary septum, with forerunners dividing the posterior left cusp and the anterior part of the posterior right cusp; a left coronary artery between the left forerunner and the posterior commissure; a right coronary artery in the restricted region between the right forerunner and the ridge indicating division of the right posterior cusp; a corresponding decrease of the posterior (aortic) part of the trunk orifice, and of the small posterior part of the trunk, which gave off the innominate and left carotid arteries, then went as a narrow (2 mm.) aortic arch to join a large ductus; this and two normal pulmonary arteries derived from the anterior part of the trunk; the left subclavian given off at junction of the ductus with the arch; an inter-ventricular septal defect at the base of the left posterior cusp; below it a powerful muscle bundle running from the septum to the wall of the right ventricle (trabecula?); a poorly developed "crista supraventricularis."

This case appears to be a combination of partial persistence of the common trunk, with stenosis of the aortic element of the trunk and of the distal arch. There is a compensatory enlargement of the pulmonic part of the trunk and of the ductus. The arrangement of the forerunners of the aortic-pulmonary septum and of the coronary arteries would place the small fourth, noncoronary cusp but a little short of its normal position. This would indicate a degree of detorsion less than in Feller's cases 1 and 4 and mine, and not far from that in Santa Cruz' case, which this case most closely resembles in degree of septum formation. There is interesting confirmation of this in the published photograph. Beneath the septal defect, as in my specimen, is a Y-shaped septal muscle band which resembles the similar structure in my case, even to the relationship of the small papillary muscle of Lancisi. The chief point of difference is that a thin, rounded muscle passes in the same direction as the normal arch of the tricuspid ledge. In my case, involving a greater detorsion, the basal part of the diverging limb of the ledge had disappeared. It seems likely that Feller's references to a small "crista" apply to the arch of the ledge.

Feller's <sup>17</sup> case 3 (in many details this case is very unlike the preceding cases) presented: a very large bulging arterial trunk; three semilunar cusps, right posterior, left posterior and anterior; an indicated division of the right posterior cusp, as in the preceding case; a "left" coronary ostium from near the posterior commissure of the left posterior cusp; a "right" coronary ostium just anterior to the ridge dividing the right posterior cusp; absence of a trunk septum; just proximal to the arch, from opposite sides of the trunk, two arteries that enter the right and the left lung in the normal positions of pulmonary arteries; absence of the ductus arteriosus; a normal arch and descending aorta; an interventricular defect unlike the preceding one; beneath the semilunar cusps a powerful muscle arch, forming the upper septum; below its concave lower margin, a defect, bordered below by a muscular septum; wide open foramen ovale primum and secundum; persistence of the left superior vena cava; a common arterioventricular ostium, with a three-cusped valve.

The analysis of this case is difficult. It probably represents a complete persistence of the common trunk, while the high origin of the pulmonary arteries indicates a complete failure of descent, as well as of fusion, of the aortic-pulmonary spurs. A possible clue to the nature of the anomaly is found in Spitzer's <sup>8</sup> analysis of the anomaly of "mixed transposition." Furthermore, Feller's description of the septum in his case shows a considerable similarity to the septum of a specimen illustrating this anomaly, which I have studied. The chief difference is that in his case the solitary trunk rose exclusively from the right ventricle, and the only communication with the left was through the low septal defect, while in my specimen the two trunks rose from opposite sides of the heavy muscular septum, with a low defect. Spitzer's interpretation is that the apparent septum was really a pseudoseptum, resulting from

the fusion and hypertrophy of the crista and the tricuspid ledge, which appeared with extreme detorsion.

Feller's own interpretation is that the small indicated right posterior cusp was the noncoronary cusp, a position that would indicate normal torsion. A much more probable conclusion is that the anterior cusp was the noncoronary cusp, and that there was almost complete (180 degrees) detorsion. One would also have to assume a transposition of the coronary arteries, the apparent left being the right, and the apparent right, the left. And further there was an indicated restriction of the pulmonic part of the ostium, a common phenomenon in detorsion defects. It is interesting that such a restriction should be indicated prior to descent of the aortic-pulmonary septum. There is confirmatory evidence in favor of a severe detorsion defect and of the foregoing theory in the various malformations at the venous end of the heart.

*Group C. Possible Cases of Persistent Common Trunk with Three Semilunar Cusps.*—From all of the evidence given, from the occurrence of cases such as those in the preceding group, which show a transition from four to three cusps, and from the occurrence of cases such as Rokitsky's, with three cusps and a well developed but incomplete trunk septum, it is evident that there may be numerous examples of persistence of a common trunk, with but three cusps. In the reported cases the evidence is often far from complete, particularly in the instances in which the blood reaches the lungs only through collateral channels. I have found but five probable and two other possible cases, in which the common trunk is identified by its giving off of true pulmonary arteries.

Grant's <sup>37</sup> case was that of a mulatto girl 16 years old. The observations were: a solitary trunk with three semilunar cusps, posterior, and right and left anterior; two coronary arteries, both from the right anterior sinus of Valsalva; two arteries to the lungs, leaving the trunk at the pericardial attachment, and a defect in the interventricular septum. No other abnormalities were described.

Siegmund's <sup>28</sup> case 2 was that of a girl 12 days old. The observations were: an almost bilocular heart, with only a rudimentary interventricular septum; absence of atrial septums; a common atrioventricular opening; a trunk with three cusps; two coronary arteries from the sinuses of the "right and left" cusps; two arteries arising independently 1 cm. above the semilunar cusps and entering the lungs in the manner of pulmonary arteries; no trace of an aortic-pulmonary septum or ductus arteriosus; a normal arch and aorta.

Klemke's <sup>33</sup> was that of a laborer, aged 25 years. The observations were: a trunk with three cusps, anterior, and right and left posterior; two coronary arteries, arising from the sinuses of the anterior and posterior left cusps; two arteries arising from the posterior left part of the trunk, a short distance above the cusps, and entering the lungs like pulmonary arteries, the margins of the ostia of which appeared as two convex arches, the lateral ends lost on the arterial wall, the medial fused (i. e., an interpulmonic spur formed); absence of ductus arteriosus; a normal arch and descending aorta; an interventricular defect in the "anterior and middle thirds" of the membranous septum; the trunk in a "rider" position; slight patency of the foramen ovale.

Wirth's<sup>12</sup> case was that of a boy who died one hour post partum. The observations were: three semilunar cusps, anterior and right and left posterior, thick at the margins; two coronary arteries from the posterior right and the anterior cusps; an artery leaving the trunk 1.5 cm. above the cusps and entering the left lung in the position of a pulmonary artery; absence of a right pulmonary artery and of a ductus arteriosus; two bronchial arteries to the right lung, from the descending aorta. Other features were in general like those of cases in groups A and B.

Dickson and Fraser's<sup>35</sup> case was that of a boy 4 months old. The arrangement of the cusps (and coronaries?) was as in Wirth's case. The only important difference was the presence of the right as the solitary pulmonary artery, and of bronchial arteries to the left lung.

The most probable explanation of the cases in this group is that they represent a complete persistence of the common arterial trunk. As far as is known, both coronary arteries are never transposed to the pulmonic trunk, so the alternate identification of the cases as examples of aortic atresia is very unlikely. The identification is most complete in Klemke's case, in which an interpulmonic spur had developed. It is interesting, too, that the patient in this case was the only one in the entire series who survived to adult years. This fact and the clinical history indicate that, clinically, the defect was not unlike pulmonic atresia. It is also of interest that the last two cases form a transition between those of complete common trunk with two independent pulmonary arteries, and the group with only bronchial arteries.

Wenner's<sup>31</sup> case 5 was that of a girl 3 days old. The "pulmonary" arteries were described as originating high. The one to the right lung rose opposite the right innominate; that to the left, a little higher. No description is given of the manner of entrance to the lungs. The general features were like those in groups A and B. This information makes it highly probable that these arteries were bronchial instead of pulmonary arteries.

Wenner's<sup>31</sup> case 6 was that of a boy 2½ days old. The observations were: a monoventricular heart with a small left atrium, a wide foramen ovale, a persistent left superior vena cava and atresia of the atrioventricular orifice; two pulmonary arteries given off from the wide arterial trunk; only one coronary artery.

From the information given, I do not believe that it is possible to classify this case exactly. Also, there is a resemblance of its main features to those of von Konstantinowitsch's<sup>20</sup> specimen. It is possible that this case, too, may represent a solitary pulmonic trunk, with a misplaced coronary ostium.

The remaining cases are all examples of single arterial trunks, with no recognizable derivatives of the sixth arch (Hülse,<sup>14</sup> Zimmermann,<sup>34</sup> Siegmund<sup>28</sup> [case 1]. Finley<sup>26</sup> and Wright and Drake<sup>32</sup>). The blood supply to the lungs was not known in the last case, and a small vessel from the concavity of the aortic arch may have been either a ductus arteriosus or a bronchial artery. One outstanding feature is that in the first four of these cases the patients had lived for periods of from eighteen to thirty-three years. The possibility of disappearance of

atretic remnants that might have been recognizable in infancy can not be excluded. I believe that on available evidence the positive identification of these cases and those of Wenner as instances of common arterial trunk is impossible. But it is also possible that a careful study of the general cardiac structure, and especially of the muscular structures of the right ventricle, as interpreted by Spitzer, may give criteria for positive identification.

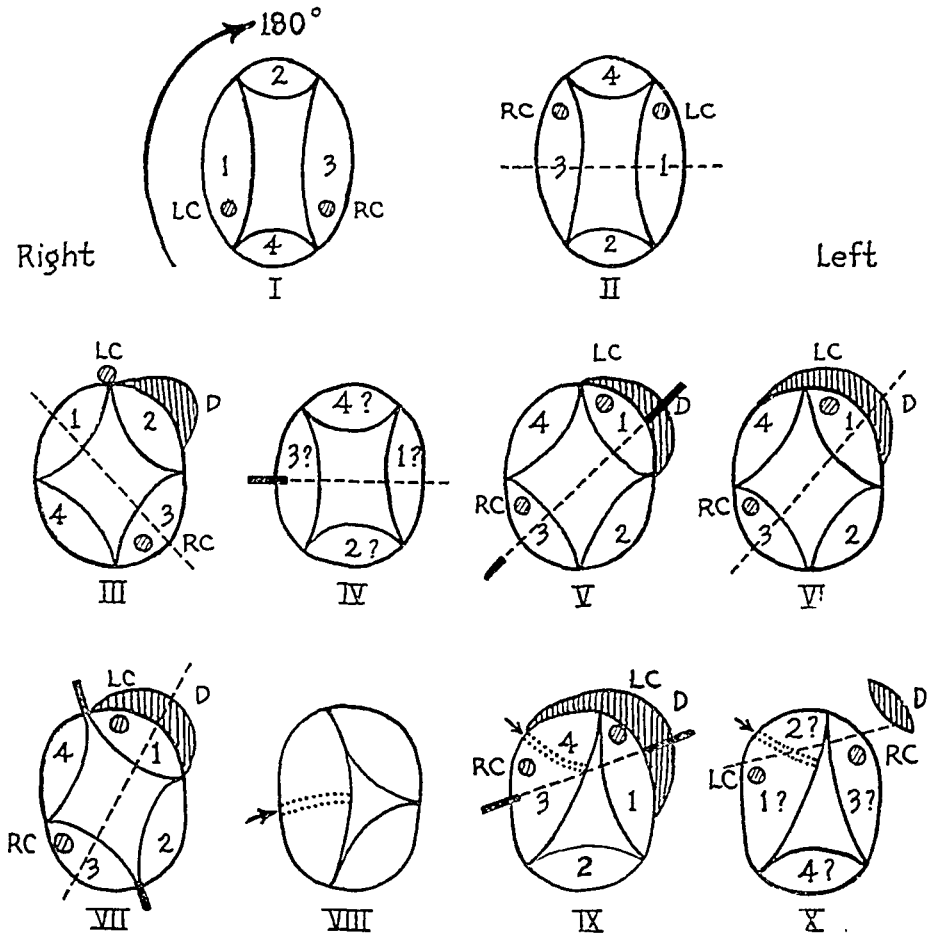


Fig. 7.—Diagrams showing the arrangements of the cusps in the reported cases of common trunk: *I*. Normal arrangement before rotation. *II*. Normal arrangement after torsion of 180 degrees. *III*. Preis's case. *IV*. Santa Cruz's case. *V*. Feller's case 1. *VI*. Feller's case 4. *VII*. Case reported in this paper. *VIII*. Buchanan's case. *IX*. Feller's case 2. *X*. Feller's case 3. The dotted line indicates the theoretical position of the trunk septum. The heavy lines show the position of septal ridges, where present. The double dotted lines show the rudimentary subdivision of a cusp. *D* indicates a defect in the interventricular septum, and *L. C.* and *R. C.*, the position of the left and right coronary ostia.

#### CRITERIA FOR IDENTIFICATION OF THE COMMON ARTERIAL TRUNK

From a study of the developmental factors concerned it is obvious that there is no one typical picture of the common arterial trunk. How-

ever, from a study of these factors and of the features of a series of cases illustrating different types of this anomaly the general characteristics can be formulated.

1. The primary requirement is that only one large arterial trunk leaves the base of the heart. The possibility of an atretic companion must be excluded by a careful search for a small vessel or a fibrous cord in regions where such remnants might be expected to persist. The size of the trunk should approximate the size of the two arterial trunks combined. Obviously this requirement cannot be rigidly enforced. Size may be modified by decrease (stenosis) of the realm of one of the arteries, while a solitary trunk may reach a considerable size in compensating for its atretic companion.

2. The arterial trunk must combine the features and functions of both the aorta and the pulmonary artery, on the one hand giving off the coronary and systemic arteries, and on the other supplying blood to the lungs. When the sixth arch structures fail to develop or when they regress early, the arterial blood must reach the lungs through collateral channels. When the sixth arch spurs are present but fail to fuse to form a septum, the pulmonary arteries rise independently from the ascending trunk. In either of these cases, the anomaly is "total" or "complete." In the "partial" anomaly, a rudimentary septum delimits an abbreviated pulmonic trunk, which gives off the pulmonary arteries and occasionally a ductus arteriosus. Externally, the septum may be indicated by a groove. From within it appears as a low ridge, or a distinct arched fold, stopping at varying heights above the ostium. It frequently sends ridges as forerunners along the line of descent, and these may reach the ostium, indicating a division into aortic and pulmonary realms. These ridges may occasionally deviate from the expected lines of descent.

3. An interventricular septal defect is always present. This is commonly an opening in the septum of the cardiac bulb, anterior to a residuum of the membranous septum, i. e., in the interaortic septum of Spitzer or the posterior part of the anterior septum of Rokitansky. With extreme detorsion it may lie lower, but still in the conus septum (septum spurium); or there may be complete failure of development and fusion of the elements making up the ventricular septum.

4. The trunk, commonly in the position of the "rider" aorta, with varying degrees of shift to the right, may, with extreme detorsion, rise solely from the right ventricle. With the shift to the right, hypertrophy of the right cardiac chambers develops.

5. The reasons why one cannot demand the ideal picture relative to the location of the coronary arteries and the number of the cusps have



been stated. However, the surest landmark of the common trunk is the possession of four semilunar cusps, with two coronary arteries rising from the sinuses of opposite cusps, both shifted toward one of the interpolated cusps.

6. The crista supraventricularis proper (Spitzer) should stop at the base of the septum, beneath the ostium of the trunk.

7. The interatrial septum is defective according to the degree of detorsion at the venous end of the heart. There are varying degrees of patency of the foramen ovale (secundum), correlated with the degree of development of the limbus and the valve and with the displacement of the latter to the left. A foramen ovale primum may be present, or there may be complete absence of the atrial septum, both accompanied by a common atrioventricular opening.

8. The mitral and tricuspid leaflets undergo modifications of form and attachment, according to the degree of shift of the trunk to the right and the assumption by the trunk of the features of the "transposed" or "right" aorta.

There are no other recognized essential associated anomalies. A fairly common variant is the persistence of the right fourth aortic arch as the arch of the aorta, instead of the normal left.

#### CAUSE OF THE DEFECT

From a survey of the reported cases and a consideration of the developmental factors, it is obvious that the anomaly in question is frequently, if not invariably, associated with abnormal torsion. On theoretical grounds, one would expect to find septum formation impaired with a lag of torsion. So far as the evidence offered by the cases with four recognizable semilunar cusps goes, there is certainly a general parallelism between the degree of septum formation and the estimated torsion defect. That this parallelism is not absolute is indicated by the instances of "transposition" of the arterial trunk, with complete separation of the trunks, in spite of extreme detorsion. One must conclude that failure of development of the aortic-pulmonary septum, while almost invariably associated with detorsion, does not stand in a simple cause-effect relation to it.

From the evidence in my own case, it might be argued that the abnormality of the transection of the commissures instead of the lateral swellings by forerunners of the septum was responsible for the failure of descent. This certainly does not hold for the more numerous cases in which the indicated lines of descent were missing or were in the expected positions. Primary inequality of the fields belonging to the two trunks, perhaps associated with a missing bulb swelling, is probably

not a necessary factor. It seems likely that such inequality is responsible for some of the stenosed, often two-cusped trunks in "transposition."

There is no evidence in the anatomic structure of the lungs in the reported cases of any fundamental abnormality in the development of the pulmonary capillary bed. In these cases as in pulmonary atresia, more or less normal pulmonary function may develop, and even be maintained for years. In spite of Spitzer's theory that normal development of the pulmonary capillary bed is an important element in the evolution of the septum, that structure may be absent or rudimentary in the presence of an adequate pulmonary circulation.

In the absence of any demonstrable correlation, one can only assume an intrinsic defect in the sixth arch system, with aplasia or early regression in the complete anomaly with only bronchial arteries, and with failure of normal growth, where sixth arch structures persist.

#### SUMMARY

There is much confusion regarding the criteria for identification of the common arterial trunk, and the literature shows many disagreements regarding the nature of the defect. The theories of the manner of development of the septums of the trunk and cardiac bulb are reviewed, as well as those pertaining to some of the defects commonly associated with imperfect development of these septums. These include abnormalities of torsion and the persistence of the reptilian right aorta (Spitzer).

The case reported is one of five in which the primitive trunk was readily identified by the possession of the theoretically required four semilunar cusps. The most widely accepted cases of persistent common trunk are reviewed. In addition to the five referred to, there are three in which the trunk had three cusps and a rudimentary fourth; five probable cases in which the trunk had three cusps, which satisfy many of the requirements, and seven cases in which the trunk had three cusps, which cannot be accurately classified. Of the thirteen probable cases, five are examples of the "partial" and eight of the "complete" defect. Five of the latter are the cases with three cusps of the third group above.

In only one case (one of the same group of five) did the patient survive to adult years. The other patients all died in infancy, although a number lived for several months. In general, the symptoms resembled those of pulmonic stenosis and atresia.

On the basis of the common features of the cases, and of theoretical considerations, suggested criteria for identification of the common arterial trunk have been evolved.

No satisfactory explanation of the cause of the anomaly has been found.

Of two cases recently reported as examples of persistent arterial trunk, that of Miller and Lyon (*Am. Heart J.* **7**:106, 1931) is an example of a single three-cusped trunk, with no recognizable derivatives of the sixth arches. Tow's case (*Am. J. Dis. Child.* **42**:1413, 1931) cannot be accurately classified from the information given.

# Laboratory Methods and Technical Notes

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## REPORT ON NECROPSIES

PREPARED BY THE JOINT COMMITTEE REPRESENTING THE NEW YORK  
ACADEMY OF MEDICINE, THE NEW YORK PATHOLOGICAL  
SOCIETY AND THE METROPOLITAN FUNERAL  
DIRECTORS' ASSOCIATION

The joint committee began its work in 1930 and rendered a report on April 8, 1931, which was approved by the Council of the New York Academy of Medicine on May 27, 1931, and published in the *Bulletin of the New York Academy of Medicine* (7: 533, 1931). In accordance with the recommendation in section D, paragraph 3, of that report a continuing joint committee was designated to carry on the work of cooperation. Because of the unexpected large demand for copies of this original report the available reprints have become exhausted. The continuing committee therefore submits the present revision as a second report on necropsies, and recommends its adoption and printing in a periodical of wide circulation.

### A. DESIRABILITY OF NECROPSY

1. All agree that postmortem examination by a pathologist is desirable; first, to provide reliable recorded information concerning the cause of death and the nature of the various disease processes; second, to confirm or amend the opinions formed by the physicians during the life of the patient, so that they may serve the next patient with greater confidence and skill; third, to reveal to the physicians continually the physical changes in the interior of the body which are associated with disordered behavior during life; fourth, to provide for the advance of human knowledge concerning the nature of disease in general. It is well recognized that the practice of postmortem examination in a hospital exercises a constant influence to improve the service and to correct serious deficiencies, as well as to improve diagnosis and prevent disease.

### B. COOPERATION OF HOSPITAL AUTHORITIES AND FUNERAL DIRECTORS

1. The hospital and its medical staff have not completed their service to the family on the death of a patient. They owe to the family a further service, namely, to give an account of what has occurred, together with the most accurate possible explanation. This requires that some representative member of the family come to the hospital for a personal interview and give permission for the examination of the body of the deceased. The funeral director must recognize this relationship and should not oppose the proper efforts of the hospital authorities and the physicians in the discharge of this obligation.

2. The funeral director is particularly interested in getting into his own hands: (1) the death certificate, (2) the permit to remove the body

and (3) the body itself, so that he may prepare it in a satisfactory manner for the funeral ceremony. He must feel certain that nothing will arise to interfere with his plan and program. Unforeseen delay may require cancellation of contracts for transportation and various other services, thus increasing the expense and causing dissatisfaction. Unreasonable delay by the hospital, in its attempt to obtain permission for necropsy, is therefore objectionable to the funeral director. The conflict of interests in this connection requires mutual consideration and a spirit of cooperation on the part of all concerned. Disputes of this nature should therefore be adjudicated by a permanent joint committee on cooperation.

3. The funeral director or his agent must present to the hospital acceptable evidence that he has been authorized by the family to take charge of the body. The blank form employed for this purpose should conform with the requirements of the department of health.

4. Hospital employees, in general, must not give information to favored funeral directors or to any other unauthorized persons in regard to persons critically ill or dead in the hospital. It is proper for the chief administrative officer of the hospital, when requested by the family, to refer the selection of a funeral director to the office of the local Funeral Directors' Association, or, quite properly, to select one by rotation from an approved list in his own office. Such a selection must never be left to a minor employee of the hospital. Proof that a minor employee has offered recommendations of this sort should be followed by his instant dismissal from the service.

5. The hospital authorities should make certain that the necessary data for a death certificate, except those facts relating to the nature, progress and termination of the present illness, are entered on the record at the time of admission of the patient. Such data as date of birth and the maiden name of the mother may be obtainable only with great difficulty after death of the patient. The death certificate may be filled out by a clerk using a typewriter, leaving only the diagnosis and signature to be supplied by the physician who completes the certificate.

6. Report of a death to the medical examiner should be made in those circumstances where this is legally required, and the decision to notify this official should be made at the time of death of the patient, entirely without regard to the attitude of the relatives concerning necropsy. It is improper for any member of the hospital staff to threaten to call the medical examiner if permission for necropsy is refused. Any such procedure of threatening or browbeating may be regarded as evidence of lack of ability to handle the situation.

7. In general, the permission for necropsy should be asked for soon after the death has occurred. Often it is best to make the request at once whenever the proper relative of the deceased is present in the hospital. Reasonable consideration should be accorded to every one concerned in determining when the matter has been adequately presented and the final decision reached.

8. Arrangements should be worked out in every hospital whereby the unnecessary loss of time on the part of the funeral director may be

obviated, and the funeral director should be instructed that he will be promptly informed by telephone when the death certificate is signed and the body is ready for him.

The telephoned information in regard to the dead, particularly before a funeral director is known to have been engaged, should be given only by an executive officer of the hospital and should be carefully guarded unless the persons on the wire are personally known.

9. Interference by a funeral director with the legitimate efforts of the hospital to obtain permission for autopsy shall be regarded as a reportable grievance.

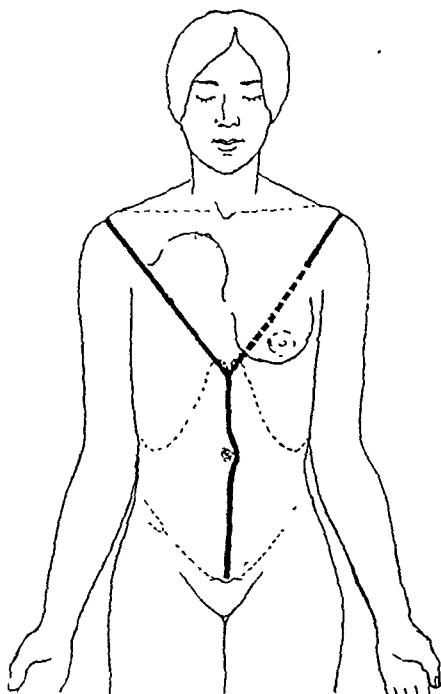


Fig. 1.—Diagram illustrating incisions in trunk.

### C. TECHNIC OF THE NECROPSY

1. In males, the incision is to extend from the suprasternal notch to the pubes in the midline, passing to the left of the umbilicus. In no circumstances shall the incision in males be extended further upward.

2. In females and in sailors who are to be buried in uniform, the V-shaped incision is to be used, that is, an incision extending from the acromial end of the clavicle to the xiphoid and up to the acromial end of the corresponding clavicle. The flap thus outlined must be dissected upward close to the deeper structures, and every effort must be made to prevent perforation of the skin in the process of dissection.

3. At least from one-half to 1 inch (1.2 to 2.5 cm.) of the external carotid arteries is to be left free and ligated. The internal carotids and the vertebrals are to be ligated, and at least from one-half to 1 inch of the iliacs is to be left intact and ligated.

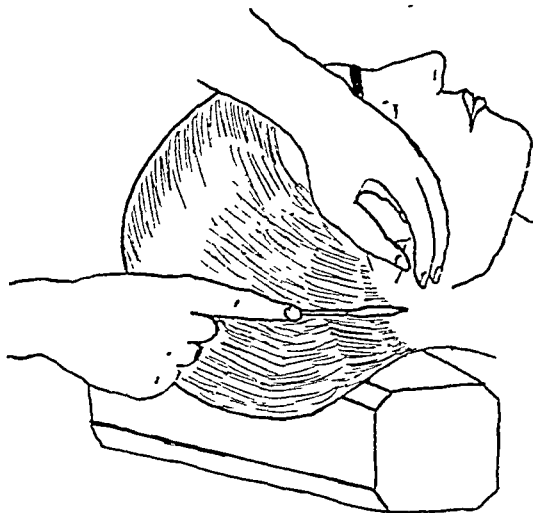


Fig. 2.—Incision in scalp.

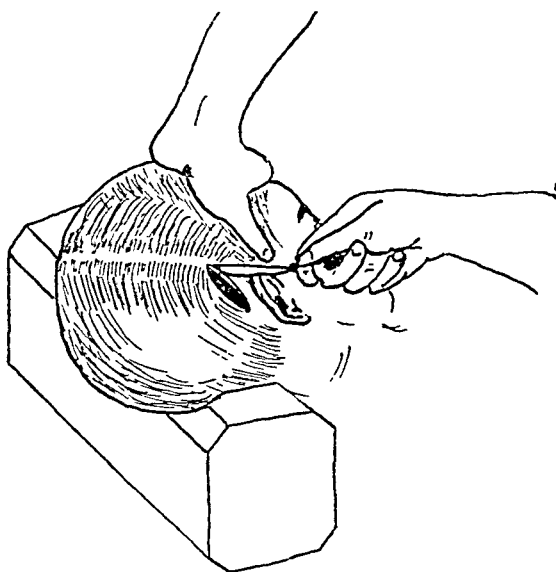


Fig. 3.—Incision in scalp.

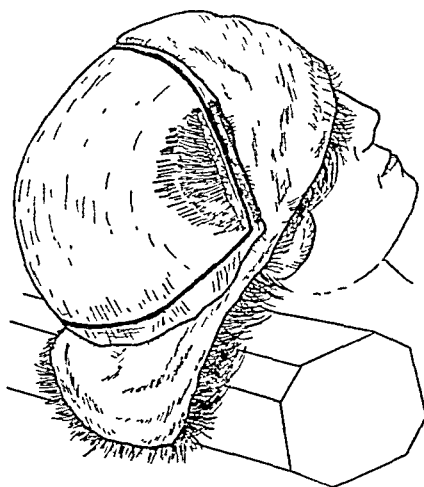


Fig. 4.—Removal of skull cap.

4. The scalp is to be divided by an incision behind the ear, extending from one mastoid process to the other, as indicated in figures 2 and 3. The incision is to pass over the vertex when the hair is abundant, or somewhat posterior to this line when it is sparse. In women, the hair is to be parted along the projected line of incision to avoid cutting it. For the same reason, after the initial incision has been made, the knife should be carried in such manner that its sharp edge faces the dissector. Care should be taken not to tear or otherwise injure the scalp. The scalp is reflected backward and forward, so that the calvarium is exposed anteriorly slightly above the frontal eminences and posteriorly somewhat behind the occipital protuberance.

Before the skull is sawed, the line through which the saw is to be carried is to be mapped out with the aid of a sharp instrument (fig. 4). The temporal muscles are to be cut on a plane parallel with the projected line (fig. 4) to preserve stumps on either side long enough to provide for suturing and immobilization of the replaced calvarium.

5. The removal of the skull cap is to be planned and carried out in such a manner as to insure its secure approximation. This is best accomplished by sawing in two intersecting lines which meet at an obtuse angle behind the ear (fig. 3), the anterior incision commencing at the level of the hair line.

6. Before closing the cranial cavity, every effort should be made to provide against leakage. This is best carried out by the following procedures: (a) by ligating the carotid and vertebral arteries, (b) by plugging the foramen magnum tightly with cotton and (c) by filling the cranial cavity with oakum.

7. In suturing the skin a moderately small needle should be used so as to avoid leakage and disfigurement.

8. After the autopsy is completed, the body is to be delivered to the embalmer in a thoroughly clean condition—the skin washed, all cavities thoroughly sponged and dried and no source of leakage allowed to remain.

9. After the completion of the autopsy, the embalmer is to be allowed the use of the autopsy room for the preparation of the body for burial, provided that this does not conflict with the immediate use of the room for another autopsy and provided also that the embalmers leave no cleaning to be done by the hospital employees.

GEORGE BAEHR, M.D., *Chairman.*



# General Review

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## CELLULAR REACTIONS OF TUBERCULOSIS AND THEIR RELATION TO IMMUNITY AND SENSITIZATION

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PHILADELPHIA

The problems concerning the pathogenesis of tuberculosis most discussed at the present time are inseparably associated with those of acute inflammation and immunity. The immediate local changes of the tissues in response to the tubercle bacillus resemble very closely the changes that follow other bacterial invasion and are identical with those that occur at the onset of inflammation produced by a great variety of irritants. The tubercle bacillus when first introduced causes scant exudation of fluid and active though transient emigration of polymorphonuclear leukocytes. The cell that later becomes predominant is a mononuclear phagocyte, which in anatomic and functional characters is similar to the mononuclear wandering cells that make their appearance whenever bacterial infection pursues a prolonged course and especially when it proceeds toward recovery. The problem of the origin of these cells is nearly half a century old, and whether they come from the blood or from the fixed tissues or from both is no better understood when their accumulation accompanies the healing of a pyogenic abscess than when as "epithelioid cells" they form the essential element of the tubercle.

The inflammatory reaction is profoundly influenced by the character of the tissue in which it occurs. The varying permeability of blood vessels in different parts of the body and the capacity of tissues to permit the accumulation of exudate are doubtless modifying factors. When a sterile irritant such as turpentine is injected into the subcutaneous tissue of a dog, an immense abscess with widespread destruction of tissue and accumulation of thick pus results, but when the same irritant in the same quantity is injected into the pleural cavity, there is a serofibrinous inflammation, which reaches maximum intensity at the end of three days and then subsides with complete restoration of the cavity to normal.

Similarly—but with due regard to peculiar characteristics of the tubercle bacillus as an inflammatory irritant—the nature of the reaction that follows its entry differs in different organs and tissues. When the disease implicates cavities that favor the accumulation of inflammatory exudates—for example, the pulmonary alveoli and the pleural, pericardial and meningeal cavities—the inflammatory nature of the reaction is generally admitted. Tuberculous pneumonia, universally recognized as a manifestation of tuberculosis only after the discovery of the tubercle bacillus, is a lesion predominately exudative and has characteristics that are reproduceable only in pulmonary tissue.

It is my belief that the use of the words exudative and productive, in the classification of tuberculous lesions, has introduced a confusion from which there is no escape save by eliminating them from nomenclature and using them only as descriptive terms. The tubercle, in accordance with the early conception of Baumgarten,<sup>1</sup> is formed, in part at least, by proliferation of cells at the site of its formation and hence has been regarded as productive. This opinion was based on the highly significant observation that the mononuclear cell that constitutes the chief cellular element of the tubercle multiplies by mitosis, but Baumgarten's deduction from this is no longer tenable. The cells and serum that accumulate in the alveoli of the lung with tuberculous pneumonia, on the contrary, are presumably exudative—that is, derived from the blood. This distinction can no longer be maintained since in the early stage of tubercle formation there is emigration of polymorphonuclear leukocytes, with other evidence of exudation from blood vessels. Moreover, mononuclear cells that accumulate in the alveoli also multiply by mitosis and assume the characteristics of those seen in the tubercle. Indeed, it is still uncertain whether the mononuclear wandering cells of tuberculous lesions are derived from wandering cells in the fixed tissue or from similar cells in the blood or from both blood cells and wandering cells of the tissues, and some still claim that they are derived from the endothelium of small blood vessels or lymphatics.

An intimate union between the fixed tissue of the affected part and the cellular elements that have accumulated under the stimulus of the tubercle bacillus is first obvious when reticular fibers, as Russakoff,<sup>2</sup> Snow Miller,<sup>3</sup> Foot<sup>4</sup> and others have shown, make their appearance. Snow Miller showed that there is no reticulum within the tubercle of the lung seven days after inoculation, whereas at the end of fourteen days, when epithelioid cells are abundant, there is a fine network of reticulum,

1. Baumgarten, P.: *Ztschr. f. klin. Med.* **9**:93, 1885; **10**:24, 1885.

2. Russakoff, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **45**:476, 1909.

3. Miller, W. S.: *Am. Rev. Tuberc.* **7**:141, 1923; *Am. J. Path.* **3**:217, 1927.

4. Foot, N. C.: *Am. J. Path.* **1**:341, 1925.

which is coarser at the periphery of the tubercle, where it is continuous with the fibers of the adjacent normal alveolar walls. Later the tubercle is wholly permeated by reticular fibers. When collagen fibrils penetrate the tubercle and tend to replace it, they represent, Miller thinks, a continuation of the same process. These are reparative changes.

It is highly probable that the prolonged course of tuberculosis with formation of epithelioid and giant cells is attributable to the insoluble fat or wax that constitutes a considerable part of the tubercle bacillus and protects it from disintegration. There is a close but by no means exact resemblance between the tubercle and the reaction of the tissues to insoluble foreign bodies. Nevertheless, it is not improbable that differences in virulence such as that between the human and the bovine bacillus depend on factors other than the presence of insoluble fat (wax) and, resembling those that determine the virulence of other micro-organisms, are as yet unfortunately not well defined.

#### THE CELLULAR REACTIONS OF TUBERCULOSIS

Inflammation may be regarded as the process by which cells and serum accumulate about an injurious substance and as far as possible bring about its destruction or removal. The phenomena of inflammation repeat themselves in the same orderly succession with a great variety of bacteria, with many other micro-organisms and with various substances, either particulate or not, soluble or insoluble in body fluids, that act as sterile inflammatory irritants. The reaction is characterized by (1) exudation of fluid and emigration of polymorphonuclear leukocytes, followed, as this first stage decreases in intensity, by (2) accumulation of mononuclear phagocytes (macrophages). It has long been known that lymphocytes and monocytes (large mononuclear leukocytes) migrate from the blood vessels into the inflamed area, but what their relation is to one another and to the mononuclear phagocytes that are so abundant in the later stages of inflammation is a time-honored controversy.

I shall not discuss in detail the older literature describing the initial reaction of polymorphonuclear leukocytes to the tubercle bacillus. The studies of Benda<sup>5</sup> on the formation of tubercles in the glomerules of the human kidney show that it occurs in man as in other animals. Polymorphonuclear leukocytes are a conspicuous element in the tuberculous tissue formed when tubercle bacilli are injected into the pleural cavity of the dog, and leukoprotease is demonstrable in this tissue (Opie

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5. Benda, C., in *Pathologisch-anatomische Arbeiten Herrn Geh. Medicinalrath Dr. Johannes Orth zur Feier seines 25 jährigen Professoren—Jubiläums gewidmet*, Berlin, A. Hirschwald, 1903, p. 520.

and Barker<sup>6</sup>). Lurie<sup>7</sup> found more polymorphonuclear leukocytes in rabbits after their infection with the bovine than after their infection with the human bacillus and more in the lung than in the liver; that is, they were more abundant with the more virulent strain and in the more susceptible organ.

The earliest stages in the formation of the tubercle were very recently described by Vorwald.<sup>8</sup> At the end of one hour after intravenous injection of tubercle bacilli, polymorphonuclear leukocytes form sharply localized collections in the alveolar capillaries. Even at this early period it is difficult to find tubercle bacilli that have not been taken up by polymorphonuclear leukocytes. By this means, the tubercle bacilli introduced into the blood stream are concentrated in small localized cellular masses, which are the ground-work for the development of tubercles. Accumulation of polymorphonuclear leukocytes is most conspicuous between the fourteenth and the eighteenth hour after inoculation. At this time, mononuclear cells are infiltrating the polymorphonuclear masses, ingesting the polymorphonuclear leukocytes and taking over the contained tubercle bacilli. After twenty-four hours, the focus of cells is predominantly mononuclear.

A second less active migration of polymorphonuclear leukocytes occurring when the tubercle undergoes caseation has been described by Kostenetsch and Wolkow<sup>9</sup> and subsequent observers. It is evident that polymorphonuclear leukocytes that penetrate into the caseous material may undergo destruction. Medlar<sup>10</sup> found evidence that polymorphonuclear leukocytes induce caseation. The pathogenesis of a tuberculous cavity is, however, very different from that of an abscess, and Medlar's designation of a tuberculous cavity as an abscess is not, I believe, well chosen.

The notable observation of Baumgarten<sup>1</sup> that the mononuclear cell that constitutes the chief element of the tubercle undergoes active mitotic division was accepted by many as evidence that the epithelioid cell is derived from fixed cells of the part affected. Increased knowledge of the wandering cells of the blood and of the tissues has modified this opinion, but has not diminished the significance of the observation, which is often forgotten.

The epithelioid cells of the tubercle have a superficial resemblance to true epithelial cells, because there is no appreciable intercellular substance between them. They have distinctive cytologic characteristics.

6. Opie, E. L., and Barker, B. J.: *J. Exper. Med.* **10**:645, 1908.

7. Lurie, M. B.: *J. Exper. Med.* **55**:31, 1932.

8. Vorwald, A. J.: *Am. Rev. Tuberc.* **25**:74, 1932.

9. Kostenetsch and Wolkow: *Arch. de méd. expér. et d'anat. path.* **4**:741, 1892

10. Medlar, E. M.: *Am. J. Path.* **2**:275, 1926.

They are large cells, and each has a large nucleus and, as Castrén<sup>11</sup> observed, a well developed cytocentrum and a large attraction sphere.

The characteristics of the epithelioid cell of the tubercle after supravital staining with neutral red and the central rosette of neutral red granules were first described by Sabin, Doan and Cunningham<sup>12</sup> and subsequently by M. R. Lewis, Willis and W. H. Lewis<sup>13</sup> and others. Neutral red granules form a rosette near the center of the cell with the nucleus just outside it. The granules are radially arranged about a clear central spot in which is the centrosome of the cell. The cytoplasm surrounding the neutral red rosette contains a varying number of fat globules, mitochondria and possibly other granules. A thin, clear peripheral zone is often extended out into short, irregular membranous pseudopodia. Giant cells similarly stained have precisely the same structure, with the nuclei arranged about a central group of neutral red granules.

Sabin, Doan and Cunningham found that some of the wandering cells of the connective tissue and of the tubercle possess a neutral red rosette like that of the monocyte of the blood, whereas others exhibit neutral red granules scattered through the cell. Carrel and Eberling<sup>14</sup> by tissue culture methods found that the distribution of neutral red granules in the mononuclear wandering cell is dependent on the functional activity of the cell, and Lewis and Lewis<sup>15</sup> found that monocytes of the blood in tissue culture produced cells with scattered neutral red granules. The opinion that variations of supravital staining identify functional phases of one type of cell seems to be widely accepted.

The evidence at hand indicates that monocytes and epithelioid cells, both with neutral red rosettes, increase in number with tubercle formation. Mononuclear phagocytes with scattered neutral red granules are constantly present in considerable number. It is not yet evident what the relation of one to the other is as they occur in the tubercle.

Sabin, Doan and Forkner<sup>16</sup> found that phagocytes with diffusely scattered neutral red granules ingest and fragment tubercle bacilli, and maintained that the micro-organism persists intact in the monocyte. On the other hand, disintegration of tubercle bacilli within monocytes of

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11. Castrén, H.: *Arb. a. d. path. Inst. zu Helsingfors* **3**:191, 1925.

12. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: *Contrib. Embryol.* **16**: 125, 1925.

13. Lewis, M. R.; Willis, H. S., and Lewis, W. H.: *Bull. Johns Hopkins Hosp.* **36**:175, 1925.

14. Carrel, A., and Eberling, A. H.: *J. Exper. Med.* **44**:285, 1926.

15. Lewis, M. R., and Lewis, W. H.: *Contrib. Embryol.* **18**:95, 1926.

16. Sabin, F. R., and Doan, C. A.: *J. Exper. Med.* **46**:627, 1927. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: *ibid.* (supp. no. 3) **52**:1, 1930.

the guinea-pig is described by Gottlieb<sup>17</sup> and in epithelioid cells of the rabbit by Lurie.<sup>7</sup> The destruction of tubercle bacilli by epithelioid cells will be discussed later.

In the absence of knowledge concerning the behavior of the neutral red rosette of the mononuclear phagocyte in the presence of various particulate and other chemical irritants in varying quantitative relations, it is doubtful if it can be successfully used as a criterion for analysis of the chemical constituents of the tubercle bacillus. It is difficult, for example, to interpret the significance of cellular reactions obtained by intraperitoneal injection of quantities of phospholipin equivalent to the content of 5 Gm. of tubercle bacilli (Sabin and Doan).

The experiments of Lurie<sup>7</sup> working at the Henry Phipps Institute have shown that different organs possess inherently different power to destroy tubercle bacilli before the phenomena of immunity or of sensitization have made their appearance. He counted the number of colonies obtainable from a weighed quantity of tissue, and found that when tubercle bacilli are injected into the blood stream of the rabbit, they, like inanimate particulate matter such as carbon particles, lodge in greatest number in the spleen, in somewhat smaller absolute numbers in the liver, though in relatively larger numbers in proportion to its volume, and in diminishing numbers in the lungs, bone marrow and kidneys. In all of these organs, tubercle bacilli multiply for several weeks. Human tubercle bacilli multiply more rapidly than bovine bacilli. With human tubercle bacilli, which are relatively avirulent for rabbits, multiplication ceases between the second and the fourth week, and with small doses, somewhat later (from the fourth to the eighth week). Subsequently the number of tubercle bacilli rapidly diminishes in all of the organs that have been named, but much more slowly in the lung, where multiplication was greatest.

With bovine tubercle bacilli, which are virulent for rabbits, as with the human micro-organisms, there is at first some but less multiplication in the spleen, liver and bone marrow, followed by progressive destruction beginning after the fourth week and completed after two months. The fate of the virulent bovine tubercle bacilli in the lung and kidney is wholly different from that of the human bacilli, since multiplication proceeds uninterruptedly and is the evident cause of death. A comparison between these changes indicating the fate of the tubercle bacilli and the histologic changes in the tissue has given new insight into the significance of the reaction that produces the tubercle. When human tubercle bacilli or bovine bacilli of an avirulent strain are injected intravenously, mononuclear wandering cells accumulate in the alveolar septums of the lung in appreciable numbers to form small groups

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17. Gottlieb, R.: *Am. Rev. Tuberc.* **25**:172, 1932.

within twenty-four hours. In an early tubercle of the lungs, at the end of a week, when tubercle bacilli are multiplying, these cells have taken on the characteristics of young epithelioid cells, the center of the nodule being composed of larger cells, each with reticulated cytoplasm and a large, round nucleus poor in chromatin. At the periphery there is a closely packed ring of smaller mononuclear cells, many of which are undergoing mitotic division. There are numerous tubercle bacilli in the young epithelioid cells. The presence of acid-fast granules in these cells indicates that tubercle bacilli are in process of destruction. At the end of two weeks, when the number of tubercle bacilli demonstrable by cultures has diminished, the epithelioid cells have assumed a more mature character and the surrounding ring of smaller mononuclear cells is very thin or absent.

While the tubercle bacilli are multiplying, young mononuclear wandering cells at the periphery of the tubercle are multiplying by mitosis to form epithelioid cells that ingest and destroy the bacilli; when the invading bacilli have been destroyed, mitosis ceases, giant cells appear, and subsequently lymphocytes and fibroblasts invade the tubercle.

It is noteworthy that mononuclear phagocytes that accumulate within the interstitial tissue of the lung and form tubercles evidently destroy the tubercle bacilli much more effectively than the similar cells that have accumulated within the alveoli. Here there may be numerous tubercle bacilli within cells with the characteristics of epithelioid cells at a time when they have disappeared from interstitial tubercles.

After intravenous injection of bovine tubercle bacilli, their multiplication in the lung, measured by cultural methods, proceeds uninterruptedly. The initial cellular reaction is more intense, the migration of polymorphonuclear leukocytes and the accumulation of mononuclear cells being much more active than after injection of the less virulent human tubercle bacilli. In interstitial tubercles, the micro-organisms are held in check, and few are found in epithelioid cells, but the pneumonic process proceeds unrestrained. Tubercle bacilli are numerous, the lesion is widespread, and caseation is extensive; new formation of epithelioid cells continues unabated to the end, and giant cell formation is inconspicuous.

Enumeration of colonies shows that tubercle bacilli disappear more readily in the liver, spleen and bone marrow than in the lungs, and histologic examination shows that acid-fast particles formed by disintegration of tubercle bacilli quickly make their appearance in the Kupffer cells of the liver, in the macrophages of the spleen and in the reticular cells of the bone marrow. The mononuclear wandering cells of different organs evidently vary in their capacity to destroy tubercle bacilli. With human tubercle bacilli, this destruction in the liver is occurring between the second and the fourth week, at a time when

mononuclear cells are multiplying by mitosis and assuming the characteristics of mature epithelioid cells. With bovine bacilli, the same process occurs somewhat later. With prompt destruction of tubercle bacilli in the liver, the whole process pursues a much more rapid course than in the lung; numerous small tubercles are formed, giant cells appear early, and caseation is inconspicuous. Between the sixth and eighth weeks, only a few bacilli are demonstrable by cultures, and tubercles of the liver, unlike those of the lung, have disappeared.

In the spleen there was histologic evidence that destruction of tubercle bacilli progressed more rapidly in the pulp than in the corpuscle, accumulation of acid-fast particles being more conspicuous in the mononuclear phagocytes of the former. Tubercle bacilli disappeared sooner, mitosis was less conspicuous, and giant cells appeared earlier in the tubercles of the pulp. In the corpuscle, mitosis of mononuclear cells persisted longer, and tubercles attained a much larger size and were present at a time when they had disappeared from the pulp.

During the period when tubercle bacilli are multiplying and accumulating within the mononuclear cells there is proliferation of these cells by mitosis, and the more active the growth of the bacilli the greater the regeneration of the cells. Mitosis is more conspicuous in the lung, which destroys the micro-organism slowly, than in the liver, in which destruction is rapid. At the time when tubercles consist of mature epithelioid cells, tubercle bacilli have already been in great part destroyed.

*Summary.*—It is now possible to formulate a somewhat more exact conception of the development of the tubercle. The mononuclear cells that first accumulate mature to form epithelioid cells. At their periphery new mononuclears are formed, in part at least, by mitotic division, and these in turn mature to form epithelioid cells. This process continues from the periphery inward as long as living tubercle bacilli persist. Caseation occurs in the center of the tubercle, and an increasing number of epithelioid cells may be involved. The fatty droplets in these cells are doubtless evidence of beginning degeneration.

The most important factor in the destruction of the tubercle bacillus is the young epithelioid cell, but available evidence suggests that the polymorphonuclear leukocyte has a part in preparing the way for it.

#### CELLULAR REACTIONS OF REINFECTION WITH TUBERCULOSIS

It would be unprofitable to review in detail observations concerning the cellular changes on reinfection by the tubercle bacillus. Significant observations, not widely known, were made by Joseph Nichols.<sup>18</sup> Rabbits were first infected with avirulent and later with virulent human

18. Nichols, J. L.: M. News 87:638, 1905.



tubercle bacilli. During the first three or four days these animals exhibited a higher temperature than controls infected for the first time with the virulent bacilli and were apparently sick. Subsequently this relation was reversed, the reinfected animals returning to normal when the controls began to decline. In the reinfected animals during the period of illness, tubercles composed of epithelioid cells appeared with much greater rapidity than in the controls; giant cells appeared as early as the third day; tubercle bacilli soon disappeared; there was no caseation, and the lesion in large part underwent resolution. In the controls, on the contrary, tubercles were formed slowly and gradually; they increased in size; tubercle bacilli persisted; caseation occurred, and giant cells did not appear until the twenty-fifth day.

By enumeration of colonies in suitably prepared cultures, Lurie<sup>19</sup> followed the fate of tubercle bacilli injected into the venous system in rabbits previously infected with tuberculosis. On reinfection with either human or bovine bacilli there was no primary multiplication in the organs such as occurred with the first infection, but destruction of bacilli began immediately after injection and proceeded far more rapidly than in the uninfected animals. This relation was very obvious in the liver, spleen and bone marrow, though determined with greater difficulty in the lung and kidney, because tubercles of the first infection persisted in considerable numbers in these organs. Scant, if any, macroscopic tuberculosis referable to the new infection made its appearance, and microscopic examination showed that an active, ephemeral accumulation of polymorphonuclear leukocytes was followed by a rapid nodular accumulation of mononuclear cells (observations in process of publication). Coincident with the disappearance of tubercle bacilli as determined by cultures, these cells matured to form epithelioid and giant cells within one week after inoculation. Young mononuclear cells undergoing mitosis were present in scant number and soon disappeared. Within two weeks in the liver and spleen and within four weeks in the lungs, the new tubercles had largely disintegrated, and after this time were rarely found.

The foregoing statements apply to rabbits in which the lesions of first infection persisted in the lungs and kidneys. When the primary lesions had almost completely disappeared, the ability of the animals to control a second infection was somewhat diminished and expressed itself by a less accelerated inflammatory reaction. The course of reinfection with human bacilli was the same, but after reinfection with bovine bacilli there were occasionally slight tuberculous lesions, far less extensive than the massive tuberculosis of first infection.

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19. Lurie, M. B.: *J. Exper. Med.* 50:747, 1929.

*Summary.*—In animals reinfected with tubercle bacilli, the initial transient accumulation of polymorphonuclear leukocytes and the subsequent formation of epithelioid and giant cells proceed more promptly and with greater intensity than in previously uninfected animals.

Tubercle bacilli are destroyed with greater rapidity in the epithelioid cells of the reinfected animals.

After destruction of the tubercle bacilli in the tuberculous lesions of the reinfected animals, the lesions may undergo partial or complete resolution.

#### LOCAL FIXATION OF TUBERCLE BACILLI WITH REINFECTION

The changes at the site of reinfection were compared with those at the site of first infection by Lewandowsky,<sup>20</sup> who introduced tubercle bacilli by scarification of the skin surface. Twenty-four hours after the second infection there was intense inflammatory edema, and leukocytes had accumulated in great numbers about the tubercle bacilli, which were present in conspicuous clumps. Necrosis of tissue had occurred about them, and polymorphonuclear leukocytes near the micro-organisms had undergone destruction. Between the fourth and the seventh day after inoculation, the necrotic tissue, carrying with it the greater part of the infecting tubercle bacilli, had been cast off, leaving an ulcerated surface; in the edges of the defect at the end of a week there were epithelioid and some giant cells, but few or no tubercle bacilli. In animals with a first infection there was fairly intense infiltration with epithelioid cells and giant cells, and tubercle bacilli were present in considerable numbers. Lewandowsky thought that the necrosis and sloughing of the dead tissue at the beginning of reinfection eliminated the greater part of the inoculated tubercle bacilli.

In this connection, the observations of Krause and Willis<sup>21</sup> on the local fixation of tubercle bacilli at the site of reinfection are significant. By inoculation of excised tissue into guinea-pigs they showed that dissemination of tubercle bacilli injected into the skin of previously infected guinea-pigs proceeds much more slowly than in guinea-pigs infected for the first time. In animals rendered both sensitive and resistant by foregoing infection, transit of bacilli from the site of entry to neighboring lymph nodes, a distance of 4 or 5 cm., required two or three weeks, whereas in previously uninfected animals it was made within twenty-four hours. They cited the local fixation of bacteria at the site of an inflammatory reaction, and believed that retardation of dissemination is the result of the heightened inflammatory reaction that

20. Lewandowsky, F.: Arch. f. Dermat. u. Syph. **123**:1, 1916.

21. Krause, A. K.: Am. Rev. Tuberc. **11**:343, 1925. Willis, H. S.: *ibid.*, p. 427.

occurs in the sensitized animal. My associate, Dr. Freund, using dead tubercle bacilli to protect guinea-pigs, obtained similar results.

This retardation of spread, Rich<sup>22</sup> suggested, may be attributable to a destruction of the bacilli referable to immunization, but he said that the heightened inflammatory reaction doubtless promotes the accumulation of cellular elements and serum containing antibodies at the site of invasion and destroys rather than fixes the bacilli. My observations<sup>23</sup> on the pathogenesis of Arthus' phenomenon, which has only partial resemblance to the inflammatory reaction occurring at the site of reinfection with tubercle bacilli, show that the foreign protein injected into the skin of an immunized animal is fixed at the site of entry and fails to reach the blood stream, whereas in a normal animal it produces scant inflammatory reaction and readily finds its way into the blood.

Pyogenic bacteria injected into the peritoneal cavity previously inflamed by an irritant do not enter the blood stream, whereas when bacteria and the irritant are introduced simultaneously, dissemination of the former is not prevented. When the fate of the tubercle bacilli introduced into the skin of an animal into which such bacilli have previously been injected is compared with the dissemination of bacteria introduced at the site of a sterile inflammatory reaction, it is significant that the tubercle bacilli themselves, like the foreign protein mentioned, incite the inflammation, evidently with the aid of some factor produced by an antigenic reaction. Antibodies, such as precipitins or agglutinins, may have a part in the local fixation of foreign protein or bacteria.

*Summary.*—The available evidence indicates that the heightened inflammatory reaction of animals sensitized by infection with tubercle bacilli promotes the local fixation of reinfecting micro-organisms.

#### CASEATION AND SENSITIZATION

The relation of caseation to sensitization, or allergy, has been much discussed. It is doubtful if any useful analogy can be found between the necrosis produced by cutaneous reinfection of a tuberculous animal (Koch's phenomenon), or the necrosis caused by tuberculin introduced into the skin of a tuberculous animal, and the caseation that affects tuberculous lesions. It is certainly true that more information about each one of these processes must be obtained before one can be satisfactorily compared with another. The local necrosis produced by the injection of tubercle bacilli into the skin of a tuberculous animal is accompanied by an acute inflammatory reaction and has little resemblance to the necrosis that affects lesions composed in considerable part

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22. Rich, A. R.: Bull. Johns Hopkins Hosp. **47**:189, 1930.

23. Opie, E. L.: J. Exper. Med. **39**:659, 1924.

of epithelioid cells. These cells, when they have reached maturity, already show degenerative changes. Some writers assume that the number of tubercle bacilli found in association with tuberculous lesions may be too small to explain the occurrence of massive caseation unless the tissue is hypersusceptible to the action of products of the micro-organism. In the absence of any method by which these relations can be measured, speculation is unprofitable. It is noteworthy, on the other hand, that the tuberculous lesions produced in animals that have previously been infected with tubercle bacilli and are in consequence highly sensitive to tuberculin as demonstrated by skin tests, may exhibit much less caseation than those of control animals infected for the first time. This relation has been observed by Dr. Freund and myself in rabbits that had received repeated injections of dead tubercle bacilli and had thus been rendered both sensitive to reinoculation and resistant to living tubercle bacilli. My associate, Dr. Aronson, infected guinea-pigs with BCG and subsequently inoculated them with virulent human tubercle bacilli. Just before these animals were killed, six weeks after the second inoculation, they reacted strongly to tuberculin. There were a few tubercles in abdominal organs, notably in the spleen; in the lungs and tracheobronchial lymph nodes there were large, firm, gray foci of tuberculosis with scant spots of caseation. In control animals with a first infection there was tuberculosis with massive caseation in the spleen, lungs and tracheobronchial lymph nodes. In reinfected animals, tubercle bacilli had resisted destruction in sufficient number to produce extensive lesions, but sensitization had produced little, if any, caseation. In Lurie's experiments, on the contrary, with tubercle bacilli virulent enough to multiply in previously uninfected or in reinfected animals, new formation of epithelioid cells proceeded uninterruptedly, and caseation was extensive.

The significant experiments of Rich and Lewis<sup>24</sup> *in vitro* show that cells of the tuberculous animal are peculiarly susceptible to injury by tuberculin. In the living animal, caseation is most likely to occur where epithelioid cells are numerous. Multiplication of tubercle bacilli with continued new formation of epithelioid cells is probably the most important factor in caseation. Under these conditions, sensitization may hasten caseation, but it may perhaps occur with no sensitization.

*Summary.*—It is by no means improbable that the extent of caseation is influenced by sensitization to products of the tubercle bacillus, but since coexisting resistance to the micro-organism or its products may at the same time hold caseation in check, definite knowledge concerning the relation of caseation to sensitization must doubtless wait for improved experimental methods.

24. Rich, A. R., and Lewis, M. R.: *Bull. Johns Hopkins Hosp.* 50:115, 1932.

Multiplication of tubercle bacilli, stimulating the new formation and maturation of epithelioid cells, is probably more important in the production of caseation than is sensitization.

SENSITIZATION AND RESISTANCE (ALLERGY AND IMMUNITY)  
WITH TUBERCULOSIS

Koch's description<sup>25</sup> of the cutaneous reaction to reinfection expresses the greater part of what is known today concerning the relation of sensitization to immunity in tuberculosis. In a guinea-pig infected with tuberculosis for the first time by injection into the skin, the cutaneous lesion appears slowly and persists until death, but in a previously infected animal an intense inflammatory reaction associated with superficial necrosis produces an ulcer that quickly heals without infection of the neighboring lymph nodes.

It is evident why some of those who repeated Koch's experiment failed to obtain the same result. Kalbfleisch<sup>26</sup> did not find the reaction any more intense in rabbits that had previously been given a small infecting dose of tubercle bacilli than in normal animals, but in animals that had been given injections of larger quantities Koch's phenomenon was readily reproduced. In agreement with this observation, Roemer and Joseph<sup>27</sup> had found that a feeble tuberculin reaction was obtained in guinea-pigs a month and a half after their inoculation with small quantities of tubercle bacilli, but that after their inoculation with larger quantities a more intense reaction appeared within twenty-three days.

Sensitization as related to the tubercle bacillus is measurable (1) by reinoculation of living tubercle bacilli (Koch phenomenon), (2) by tests with tubercle bacilli killed by a variety of agents, (3) by tests with extracts obtained by methods that preserve intact the protein contents of the tubercle bacillus and (4) by tests with extracts in which the protein and doubtless other constituents of the micro-organism are profoundly modified. The best example of the latter is old tuberculin, which in preparation is subjected to prolonged heat at boiling temperature.

Information concerning sensitization obtained by one of these methods is not transferable to another. Tuberculo-protein, like other foreign protein, as Baldwin<sup>28</sup> and others have found, acts as an antigen, sensitizes to anaphylactic shock and, as its recent use in relatively large doses has shown, causes "anaphylactic" inflammation similar to that produced by other foreign protein in a sensitized animal (Arthus' phenomenon). Old tuberculin, on the contrary, is not an

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25. Koch, R.: *Deutsche med. Wchnschr.* **17**:101, 1891.

26. Kalbfleisch, H. H.: *Beitr. z. Klin. d. Tuberk.* **70**:465, 1928.

27. Roemer, P. H., and Joseph, K.: *Beitr. z. Klin. d. Tuberk.* **14**:1, 1909.

28. Baldwin, E. R.: *J. M. Research* **22**:189, 1910.

antigen in the usual sense, but serves to manifest reactions that it cannot incite.

Rich<sup>29</sup> stated that tuberculous animals desensitized by repeated injections of tuberculin are still resistant to a new infection with tubercle bacilli. Aronson, however, has found, he informs me, that tuberculous animals desensitized to tuberculin still exhibit the Koch phenomenon. Such experiments involve quantitative relations that must be more fully investigated. Existing criteria of sensitization are necessarily vague.

In the attempt to define the relation between sensitization and immunity to tuberculosis, the difficulties of measuring immunity are equally great. The criteria available are resistance to reinfection (always relative, and experimentally determined by prolongation of life or by percentage of recovery) and formation of antibodies, which may have little if any obvious relation to protection against this disease. Sensitization is a phenomenon readily demonstrable in association with some infectious diseases and inconspicuous if it occurs with others. There is no evidence to indicate that it is essential to protection. Experiments directed to the protection of cattle with the bovovaccine of von Behring showed that the tuberculin reaction and protection against reinoculation were lost after a year or a year and a half, and there is a good deal of evidence that human beings lose their susceptibility to tuberculin with complete recovery from infection. With reinfection, the formation of antibodies occurs more rapidly than after first infection,<sup>30</sup> and there is some evidence that the tuberculin reaction in human beings lost after first infection reappears with unusual rapidity after reinfection. These observations vaguely suggest that there are factors involved in immunity against tuberculosis independent of sensitization and of the formation of antibodies and even of the usual manifestation of resistance.

*Summary.*—Sensitization is inseparably associated with immunity, but since each is dependent on factors peculiar to itself, they do not necessarily proceed parallel.

#### RELATION OF SENSITIZATION (ALLERGY) TO HUMAN TUBERCULOSIS

The evident symptoms and profound injury to tissue that are produced in sensitive animals by products of tubercle bacilli support the assumption that hypersusceptibility to these products plays an important part in the symptomatology and pathogenesis of human tuberculosis.

29. Rich, A. R.: *Proc. Nat. Acad. Sc.* **16**:460, 1930.

30. Mudd, S.; Lucké, B.; McCutcheon, M., and Strumia, M.: *J. Exper. Med.* **49**:779, 1929. McCutcheon, M.; Strumia, M., and Mudd, S.: *ibid.*, p. 797. McCutcheon, M., and others: *ibid.*, p. 815.

The number of publications in which this fascinating subject is discussed is very great, especially in Germany, where interest in it has been stimulated by the important anatomic observations and highly speculative writings of Ranke.<sup>31</sup> Unfortunately there is little agreement on what may be regarded as evidence of sensitization, or allergy.

Three stages in the development of human tuberculosis, according to Ranke, are comparable with those of syphilis. The first change following the lodgment of the tubercle bacillus in the lung is, he said, a pneumonic process, and the first histologic evidence of altered reaction is tubercle formation. The second stage of infection, characterized by heightened susceptibility, is associated with dissemination of the infection by the blood stream. The third stage, or stage of immunity, is best illustrated by slowly progressive pulmonary tuberculosis, with which spread is chiefly by way of tubular channels, such as the bronchi.

Few pathologists accept the opinion that tuberculosis acquired in childhood progresses through the stages described by Ranke, but among those who regard the phthisis of adults as a reinfection acquired by those infected in childhood, opinions concerning the significance of sensitization differ widely. Aschoff,<sup>32</sup> like Ranke, regards the dissemination of tuberculosis in childhood as an anaphylactic phenomenon accompanied by exudation and rapid caseation affecting lymph nodes especially. Others, including myself, believe that dissemination by way of the lymphatic system and blood stream is the usual manifestation of tuberculous infection in susceptible animals unprotected by a previous infection. Tuberculosis of reinfected animals and the usual pulmonary tuberculosis of adult human beings are characterized by scant, if any, caseation of adjacent lymph nodes and are rarely accompanied by blood stream dissemination (Ranke, Opie,<sup>33</sup> Aschoff,<sup>34</sup> Schurmann<sup>35</sup>).

Those who have studied the primary deposition of tubercle bacilli in the tissues agree that the first changes are inflammatory. Prudden<sup>36</sup> produced massive caseous pneumonia by injecting tubercle bacilli into the bronchi of rabbits. Bezançon and di Serbonnes<sup>37</sup> found that the first effect of the introduction of tubercle bacilli into the lung of a normal guinea-pig by way of the trachea is an alveolitis with congestion of blood vessels and accumulation of mononuclear cells with some

31. Ranke, K. E.: *Deutsches Arch. f. klin. Med.* **119**:201 and 297, 1916; **129**:224, 1919.

32. Aschoff, L.: *Lectures on Pathology*, New York, Paul B. Hoeber, 1924, p. 34.

33. Opie, E. L.: *J. Exper. Med.* **25**:855, 1917; **26**:263, 1917.

34. Aschoff, L.: *Verhandl. d. deutsch. Gesellsch. f. inn. Med.* **33**:13, 1921.

35. Schurmann, P.: *Beitr. z. klin. Med.* **68**:723, 1928.

36. Prudden, M.: *New York M. J.* **60**:1, 1894.

37. Bezançon, F., and di Serbonnes: *Ann. de méd.* **1**:149, 1914.

polymorphonuclear leukocytes, followed by caseation. When tubercle bacilli were introduced into the tracheas of guinea-pigs shown by skin tests to be sensitized by previous infection, there was alveolar inflammation, but no caseation, and the lesion soon assumed the character of chronic interstitial pneumonia.

*Summary.*—Experimental evidence shows that sensitization to products of the tubercle bacillus modifies the character of tuberculous lesions and increases the intensity of the inflammatory reaction to the tubercle bacillus, but there is as yet scant information by which the varying character of human lesions can be more exactly interpreted as manifestations of sensitization.

The assertion frequently made that frank tuberculous pneumonia is never the result of a first infection is, I believe, incorrect. It is not improbable that the extent of a tuberculous pneumonia may be increased by sensitization of the tissues, but experimental and human evidence indicates that massive caseous pneumonia is more likely to occur in the absence of previous infection.

The apical tuberculosis of adults has the characteristics of tuberculosis in animals made resistant by previous infection. It usually pursues a chronic course, remains localized in the lung and is unaccompanied by caseation of adjacent lymph nodes.



## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—T. D. Beckwith has been appointed associate professor of bacteriology in the University of California at Los Angeles.

Martin Frobisher has been appointed associate in epidemiology in the Johns Hopkins School of Hygiene and Public Health, Baltimore.

In the school of medicine of the University of Texas at Galveston, Wendell Gingrich has been appointed professor of bacteriology and preventive medicine, and William M. Powell instructor in pathology.

Lyman L. Daines, professor of pathology and bacteriology in the school of medicine of the University of Utah, has assumed the deanship of the school.

Maurice Nicolle, bacteriologist and professor in the Pasteur Institute in Paris, has died at the age of 70.

In the medical college of Cornell University in New York, James Ewing is professor of oncology; Lawrence W. Smith, assistant professor of pathology, and Fred W. Stewart, Jacob Furth and Jules Freund, associates in pathology.

**Society News.**—The American Association of Pathologists and Bacteriologists will meet in Washington, D. C., on May 9 and 10, 1933, in conjunction with the Congress of American Physicians and Surgeons.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

LOCALIZATION OF EXPERIMENTAL VENTRICULAR MYOCARDIAL LESIONS BY THE ELECTROCARDIOGRAM. J. H. CRAWFORD, G. H. ROBERTS, D. I. ABRAMSON and J. C. CARDWELL, *Am. Heart J.* **7**:627, 1932.

Electrocardiographic changes were studied in relation to the site of damage in thirty-four cats in which localized ventricular myocardial lesions had been produced by the electric cautery. Curves of monophasic type were obtained, which were classified as of the  $T_1$  and  $T_3$  types of Parkinson and Bedford. With almost complete consistency, lesions in similar sites produced the same type of curve. Lesions on the anterior surface of the left ventricle produced curves of the  $T_1$  type, while those on the posterior surface of the left ventricle, including the apex, yielded the  $T_3$  type. All right ventricular sites, except the base anterior, in which only a slight change was induced, gave curves of the  $T_3$  type. At the apex posterior alone were the changes comparable in magnitude with those obtained in the left ventricle. Usually the changes produced were marked in two leads. In some the displacement of the R-T segment was oppositely directed in the remaining lead, while in others no significant deviation was observed in this lead. In a few instances, an R-T elevation was present in all three leads, but as a rule to a greater extent in one lead. In three experiments, in each of which the lesion was located at the base anterior, depression rather than elevation of the R-T segment occurred.

AUTHORS' SUMMARY.

EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM AND OSTEITIS FIBROSA IN PUPPIES. JOSEPH L. JOHNSON, *Am. J. M. Sc.* **183**:761 and 769, 1932.

Parathormone in doses of from 10 to 20 units was injected daily for periods of from ten to forty-three days into white rats, aged from 6 to 12 weeks. The rats were fed the Steenbock diet for normal rats. Control litter mates remained well, whereas the animals given parathormone, without exception, showed muscular weakness, hypotonia and skeletal lesions characteristic of osteitis fibrosa osteoplastica (von Recklinghausen), namely, a lacunar resorption of bone with softening and deformity, bending and multiple fractures. In the affected bones, the cortex and marrow were largely replaced by fibrous connective tissue containing numerous giant cells, and new osteoid tissue was also in evidence in numerous cases. Chronic hyperparathyroidism produced in rats by repeated injections of parathormone leads to bone changes that justify a diagnosis of osteitis fibrosa. These experiments support the conclusion that the cause of clinical osteitis fibrosa osteoplastica (von Recklinghausen) is an excess of the parathyroid hormone.

The experimental production in puppies of a state of chronic hyperparathyroidism is reported, and descriptions are given of the gross, roentgenologic and microscopic abnormalities resulting therefrom. This experimental disease, produced with repeated injections of parathormone, is characterized by skeletal lesions and other abnormalities which correspond closely to those observed in clinical cases of osteitis fibrosa osteoplastica.

AUTHOR'S SUMMARIES.

EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM AND EFFECTS OF IRRADIATED ERGOSTEROL. JOSEPH L. JOHNSON, *Am. J. M. Sc.* **183**:776, 1932.

An answer was sought to the question as to the relationship of osteitis fibrosa to those other skeletal diseases, especially rickets and osteomalacia, that are also

associated with hypertrophy of parathyroid glands. Metabolic studies of the problem have been reported; the results of animal experiments are given here. Viosterol, in a dosage of from 5 to 60 drops daily, was fed to young, white rats and puppies maintained in a chronic state of hyperparathyroidism by the daily injection of parathormone. These animals were litter mates of others that developed the skeletal lesions of osteitis fibrosa when treated with parathormone alone. The technic of experimentation, the diets and the dosage of the parathormone were the same as previously reported. The outcome was the same, except that the resulting lesions typical of the skeletal abnormalities of osteitis fibrosa were, on the whole, more extensive. Metastatic calcification in the kidneys was also more marked. Whereas vitamin D concentrates effectively protect against rickets or osteomalacia, it is evident from these experiments that they intensify the disease produced by excess of parathyroid hormone. It is therefore clear that rickets and osteomalacia differ essentially in pathogenesis from osteitis fibrosa. While the hypertrophy of the parathyroid glands in conditions of vitamin D deficiency may be compensatory and is certainly a secondary phenomenon, the tumors of parathyroid glands found in association with osteitis fibrosa are of primary significance. It is recognized, however, that overfunction of parathyroid glands may occur in the absence of tumors or other morphologic abnormality.

AUTHOR'S SUMMARY.

EXPERIMENTAL CHRONIC GASTRIC ULCERS IN RABBITS. A. N. FERGUSON, Arch. Int. Med. 49:846, 1932.

A method for the consistent production of experimental chronic gastric ulcers in rabbits is described. An incision was made in the anterior wall of the stomach at about the junction of the body of the stomach with the pyloric portion. This original incision extended through the serosal and muscular coats down to the submucosa and underlying mucosa, but not through these layers. One edge of the muscular coat was then lifted up, and undercutting was performed through the submucosa for about 2 cm. A circular piece of the exposed mucosa, at least 1.5 cm. in diameter, was then excised by means of scissors, after which the edges of the original incision in the muscular coat were sewed together. The primary mortality from these operations was rather high, death being due to perforation of the stomach at the site of the ulcer, perigastric abscess or incidental infection. A series of lesions (eighteen) was obtained ranging in age from 95 days to 2 years, 1 month and 17 days. A variation in the reparative powers of rabbits with chronic ulcers was noted. In some the ulcers tended to remain chronic, while in others various degrees of healing, even resulting in complete repair, occurred. Experimental chronic ulcers in rabbits tend to decrease in size, when the destructive processes are not too great, even though there is no regeneration of epithelium over the surface of the ulcer. Epithelium forming the margin of a chronic ulcer is composed entirely of foveolar cells. These cells constantly attempt to regenerate and repair the ulcer defect, and are successful when a floor suitable for their growth is formed. Foveolar cells are responsible for regeneration of the epithelium in both acute and chronic experimental ulcers. The essential factor that delays healing and produces chronicity in ulcers is that of destructive forces acting on the floor of the base. There is a constant struggle between these destructive forces and reparative processes. The outcome depends on which one is in excess and gains control.

AUTHOR'S SUMMARY.

THE TESTICULAR HORMONE CONTENT OF TISSUES AND HUMAN URINE. E. B. WOMACK and F. C. KOCH, Endocrinology 16:267 and 273, 1932.

The method of McGee, Gallagher and Koch for extracting the testicular hormone appears to be the best available at this time. Attempts to obtain active extracts from wheat, yeast, spinach and carrots were unsuccessful. The content

of hormone in the testes of rams varied more than that in the testes of bulls. The hormone has been demonstrated in the testes of fetal bulls. The hormone is present in the urine of men, adolescent boys and normal and pregnant women.

THE HEMOLYTIC ACTIVITY OF THE SPLEEN IN HEMOLYTIC ANEMIA. W. CEELEN, *Beitr. z. path. Anat. u. z. allg. Path.* **86**:175, 1931.

The author believes the site of hemolysis in the spleen to be the intersinusoidal pulp tissue, which in hemolytic anemia is so stuffed with red blood corpuscles as to appear infarcted. The sinuses are compressed and contain few erythrocytes. Many contain numerous irregularly sized, colorless spherical granules, which give the iron reaction and appear to be intermediate hemolytic products. The constitutional inferiority of the erythrocytes in this disease combined with the prolonged stay in the choked intersinusoidal pulp, due to compression of the sinusoids, favors hemolysis.

W. S. BOIKAN.

EUNUCHOIDISM IN MAN. K. LÖWENTHAL, *Beitr. z. path. Anat. u. z. allg. Path.* **86**:426, 1931.

Eunuchoidism is a definite constitutional anomaly the anatomic basis of which is an atrophy of the testicles that commences at puberty and proceeds to the extremest grade. The secondary changes in the body commence simultaneously and are dependent on the testicular change. It is apparently a chromosomal anomaly which is inheritable.

W. S. BOIKAN.

FATAL SENSITIVITY TO SUNLIGHT CALLED FORTH BY ENTERAL PORPHYRIN. L. HARANGHY, *Centralbl. f. allg. Path. u. path. Anat.* **54**:161, 1932.

A 6 year old girl who had a very slow convalescence from measles was suspected of having tuberculosis and was given sun baths for one whole summer. The following spring these baths were resumed, but the child felt so ill after a short exposure that she was taken indoors and promptly felt somewhat better. An unusually severe redness of the exposed parts occurred shortly, and a cough developed. This was in turn followed by icterus and evidences of renal injury. Ten days after exposure to sunlight the child died, and a strain of Flexner's bacillus was cultured from the bowel. This organism was found to form porphyrin in hemoglobin-bouillon mediums. The intestinal bacteria were thus suspected of having formed this substance in the body, and the porphyrin led to a strong photodynamic sensitivity. Exposure to sunlight under these circumstances resulted in marked toxemia. The author cautions against the use of quartz lamps or sunlight in treatment of persons with distress of the bowel and advocates testing the stool for porphyrin.

GEORGE RUKSTINAT.

RELATION OF GENERAL FIBROUS OSTEITIS TO THE PARATHYROID GLANDS. F. W. WICHMANN, *Deutsche Ztschr. f. Chir.* **235**:619, 1932.

In a woman, 45 years old, with general fibrous osteitis, removal of an adenoma of the parathyroid gland located in the left lobe of the thyroid gland, which was slightly enlarged, was followed by improvement in the condition of the skeleton and a return of the calcium metabolism to normal.

### Pathologic Anatomy

THE PATHOLOGIC BASIS OF SYMPTOMS IN NEPHRITIS. J. P. SIMONDS, J. A. M. A. **98**:803, 1932.

Two types of nephritis can be differentiated: (a) that in which the essential damage is to the secretory portion of the kidneys, and (b) that in which the smaller arteries and arterioles are characteristically involved. This second type

is not so much a primary disease of the kidneys as a generalized disease of the arterioles of the body.

The basic changes in the first type are degenerative and exudative (inflammatory) resulting in increased permeability of the renal filter without retention of nitrogenous products but with loss of albumin in the urine, depletion of blood serum albumin, retention of crystalloids in the tissues and edema. In the second type, the fundamental pathologic change is a hyperplastic sclerosis of the smaller renal arteries and arterioles with narrowing of their lumens, (a) reducing blood pressure and blood flow in the glomeruli below that required for adequate filtration and (b) interfering with the nutrition and therefore with the function, of the tubules, thus causing retention of nitrogenous waste products.

More elaborate classifications are confusing because they attempt to make separate entities and types out of (a) different stages of the same morbid process or (b) different combinations of the same fundamental unit pathologic lesions.

If due recognition is given to the fact that nephritis is a progressive disease, it is possible, by means of this simple classification, to make a reasonably satisfactory correlation between its clinical manifestations and their pathologic basis, and to elaborate the principles of rational treatment.

AUTHOR'S SUMMARY.

ELLIPTIC HUMAN ERYTHROCYTES. GARNETT CHENEY, J. A. M. A. 98:878, 1932.

Elliptic human erythrocytes represent a departure from the round forms usually found. Cases of their occurrence have been rarely reported. It seems probable that they are more common than the meager literature indicates. The hereditary transmission of such unusual red cell forms is emphasized by a report of a family including forty-one members in three generations, fourteen of whom show this bizarre structure in the blood. The transmission is probably by a simple mendelian dominant. Although this condition has been associated with secondary anemia and with sickle-cell anemia, there is insufficient evidence to justify assumption of a relationship. Aside from the unusual erythrocyte forms, nothing remarkable is to be noted in the blood or the bone marrow.

LESIONS OF THE CARDIAC ORIFICE OF THE STOMACH PRODUCED BY VOMITING. SOMA WEISS and G. KENNETH MALLORY, J. A. M. A. 98:1353, 1932.

Two cases of laceration and ulceration at the junction of the esophagus and the stomach resulted fatally. In the first case, the characteristic longitudinal laceration of the mucosa was acute and unusually deep, rupturing a visible artery and causing death from exsanguination. In the second case, the clinical evidence suggested that an acute laceration which had developed in the past had caused the formation of a chronic ulcer at the junction of the esophagus and the stomach. This ulcer, following an alcoholic debauch and vomiting, ruptured and perforated into the mediastinum, causing bilateral purulent empyema and subcutaneous emphysema. The concept of the mechanism involved in the development of lesions at the cardiac orifice of the stomach described in a previous communication is supported by the clinical course and postmortem observations now reported. Pressure changes in the stomach during disturbed mechanisms of vomiting, together with regurgitation of the gastric juice and the corrosive effect of alcohol, are considered to be responsible for the origin of the lesions described.

AUTHORS' SUMMARY.

HEMORRHAGE INTO THE OVARIAN STROMA IN MITRAL STENOSIS. GEORGE RUKSTINAT, J. A. M. A. 98:1716, 1932.

Rukstinat describes hemorrhagic infiltration into the stroma of both ovaries in a woman, 38 years old, who died from cardiac decompensation following mitral stenosis.

CYTOLOGIC STUDIES ON RHEUMATIC GRANULOMÀ. C. McEWEN, J. *Exper. Med.* **55**:745, 1932.

Scrapings of subcutaneous nodules from ten patients with rheumatic fever were examined microscopically after being stained with supravital dyes. From the uniform results obtained, the following conclusions have been drawn: Supravital staining of cells from these lesions gives information unobtainable with ordinary histologic methods. The scrapings show a great predominance of certain cells almost entirely devoid of phagocytic power and not characterized by the reactions with neutral red that distinguish monocytes, epithelioid cells and clasmatocytes. Hence they differ from the essential cells of the lesions of tuberculosis and experimental syphilis. These differences are probably functional and developmental rather than genetic. The cells probably arise from the undifferentiated mesenchymal elements of loose connective tissue, although it is possible that endothelial cells take part in their formation in some instances. Since there is little doubt that the subcutaneous rheumatic nodules are pathologically identical with rheumatic granulomas elsewhere in the body, these conclusions are considered applicable also to the Aschoff body cells of the myocardial submiliary nodules.

AUTHOR'S SUMMARY.

COMPLETELY HEALED DISSECTING ANEURYSM OF THE AORTA. T. SHENNAN, *J. Path. & Bact.* **35**:161, 1932.

In a man 64 years of age there were recurrent hemorrhages into and along the walls of the aorta. The original hemorrhage into the mediastinum and along the adventitia of the extrapericardial thoracic aorta, occurring about six months before admission, had organized to form a dense layer of connective tissue abutting on and buttressing the media. The point at which the escape of blood took place could not be identified. A second hemorrhage dissecting along the outer layers of the media internal to the thickened adventitia had occurred eight weeks before death, and in the interval had organized completely with obliteration of the sac. This hemorrhage originated in a rupture, now healed, of the inner coats on the wall opposite to the ligamentum arteriosum. Shortly before death a third rupture occurred on the right posterior wall in the neighborhood of the ligamentum arteriosum, and the resulting hemorrhage into the media internal to the previous organized layer formed an elongated hematoma in the wall of the descending aorta. This caused a bulging inward of the walls at the upper end of the descending aorta, producing a condition similar to isthmus stenosis, which so increased the pressure in the ascending aorta that a fourth rupture occurred proximal to the reflexion of the pericardium in the anterior wall of the bulbus. The resultant dissecting aneurysm in the wall of the ascending aorta ruptured into the pericardium and caused sudden death. There was no atheromatous change in the area of the wall involved in the proximal rupture, and none of the other ruptures was specially related to atheromatous patches or ulcers. Dissecting aneurysms originating in such ulcers are extremely rare, though this is commonly believed to be a frequent mode of origin.

AUTHOR'S SUMMARY.

THE PATHOLOGY OF ANEURYSM: A REVIEW OF 167 AUTOPSIES. H. G. GARLAND, *J. Path. & Bact.* **35**:333, 1932.

The material analyzed in this article comes from 12,000 consecutive autopsies at all ages from 1910 to 1930. The 167 cases of aneurysm are analyzed according to location and nature of the underlying lesion. Eighty-six of the cases were syphilitic; of 78 cases of aneurysm of the thoracic aorta, 91.3 per cent were syphilitic.

BONE WITHIN A RENAL CALCULUS. G. STUART and K. S. KRIKORIAN, *J. Path. & Bact.* **35**:373, 1932.

A description is given of a case of renal calculus containing living bone.

AUTHORS' SUMMARY.

ABNORMALITIES OF THE MOUSE SUPRARENAL. R. WHITEHEAD, J. Path. & Bact. **35**:415, 1932.

The abnormalities noted in the suprarenal glands of 477 untreated mice are described. They include medullary cells in the cortex, inflammatory spindle cells, cortical hypoplasia or atrophy and a hitherto undescribed type of tumor in the medulla.

AUTHOR'S SUMMARY.

THE FORMATION OF THE ASBESTOSIS BODY IN THE LUNG. S. R. GLOYNE, Tubercle **12**:399, 1931.

The absence of asbestosis bodies in crude asbestos makes it practically certain that they are produced in living tissues from inhaled asbestos fibers. Gloyne believes that they are formed in the lung by deposition of certain materials that tend to thicken the fiber, thus causing the fissure or cracking of the deposited material and giving rise to the segments. There is evidence that iron is a component of this material.

H. J. CORPER.

THE LYMPHATIC VESSELS OF THE FALLOPIAN TUBE. M. A. PELLÉ and O. PELLÉ, Ann. d'anat. path. **8**:605, 1931.

The drainage of the lymph of the fallopian tube is performed by a principal external route leading to the right and left lateral aortic ganglions, by a middle route ending in the middle chain of the external iliac ganglions and by an internal route leading to a hypogastric ganglion.

There are often anastomoses between the lymphatic vessels of the tube and the perirenal capsule, which explains the ease with which a tubal infection reaches the perirenal fat. It likewise explains the frequency of renal pain in the course of even light cases of salpingitis, and also possibly the origin of some perinephric phlegmons regarded as "primary."

The tubal lymphatic vessels have no connection with the appendix; they communicate with those of the uterus and of the ovary.

B. M. FRIED.

GENERAL CHARACTERISTICS OF MALIGNANT GRANULOMA. M. FAVRE and P. CROIZAT, Ann. d'anat. path. **8**:838, 1931.

Malignant granuloma (Favre and Croizat use this term for malignant lymphogranulomatosis) does not involve the lymphoid tissues only but more generally the mesenchyma, the supporting substances and the reticulo-endothelial system in the broad sense of this word. It spreads in a mesenchymatous medium, and the first invasive lesion may be extragranular, intradermic.

The granuloma has a peculiarly active influence on the connective tissue, "reverting" the connective tissue cells into undifferentiated elements, histiocytes and hemohistioblasts, which evolve and differentiate into granulocytes, erythroblasts and even megakariocytes. In the formation of the lesions, cells differentiated in situ play a more important rôle by far than those brought by the blood stream.

The histologic reactions appear to be inflammatory, resulting from local action of the granulomatous virus (?). The peculiar polymorphism of the lesion, its changeable and "fluctuating" aspect, is of interest when compared with neoplastic pictures which are constant. Two parallel reactions are noticed, one inflammatory and another designated as neoplastic. In fact, the only cell that appears to be neoplastic is that of Sternberg. However, even this cell is not a point of departure but an expression in the evolution of the disease. The cell should be regarded as an evolving form of the hemohistioblast. Malignant granuloma is on the borderline between inflammation and neoplastic formation. Favre and Croizat discuss in detail different pathologic and clinical aspects of the disease.

B. M. FRIED.

THE PATHOLOGIC ANATOMY OF THE HUMAN THYMUS. P. BASTENIÉ, Arch. Internat. de méd. expér. 7:273, 1932.

The disappearance of the thymocytes in the course of pathologic involution of the thymus is not the result of their emigration but of their destruction in situ. The genesis of the corpuscles of Hassall is connected with the destruction of the thymocytes. Of mesodermic origin, appearing soon after the pyknosis of the small thymic cells, the corpuscles of Hassall do not appear to have any secretory significance. Thymic involution follows any general disturbance of the body and is not peculiar to any particular disease or intoxication. So-called types of involution are disease stages of the same process. In the course of pathologic involution, the thymus presents the same elastic reactions as in physiologic involution, the indications being that in both conditions it acts as a regulator in the dispensation of nuclein.

SPECIFIC BONE MARROW CHANGES IN AGRANULOCYTOSIS. E. OPIKOFER, Beitr. z. path. Anat. u. z. allg. Path. 85:165, 1930.

Three cases of acute agranulocytosis were studied microscopically with the following results: The disappearance of the granulocytes was uniform in all organs and blood vessels. The characteristic picture of the bone marrow showed involvement of the myeloblasts in severe degenerative changes ranging to complete necrosis, absence of granulocytes and undisturbed erythropoiesis. The spleen showed a plasma cell reaction with absence of leukocytes. These results speak for an essential agranulocytosis on a toxic basis.

W. S. BOIKAN.

THE EFFECT OF POTASSIUM IODIDE ON THE THYROID GLAND OF THE RAT. F. H. IRSIGLER, Beitr. z. path. Anat. u. z. allg. Path. 85:220, 1930.

Irsigler studied the histologic changes in the thyroid gland of the rat by means of potassium iodide. Intraperitoneal administration was more active in smaller dosage than peroral. With the small dosage used by American investigators, a marked stimulation of the thyroid gland, as evidenced by numerous nuclear divisions, was obtained. With a still smaller dosage, however, Irsigler avoided obvious cellular stimulation, but obtained increased accumulation of colloid in the vesicles. True exophthalmic goiter was not produced.

W. S. BOIKAN.

CARDIAC LESIONS IN SCARLET FEVER, STREPTOCOCCUS INFECTIONS AND RHEUMATIC GRANULOMATOSIS. T. FAHR, Beitr. z. path. Anat. u. z. allg. Path. 85:445, 1931.

The focal and diffuse histiocytic proliferations in the heart first described by Fahr in scarlet fever are regarded by him as allergic reactions to the streptococci usually associated with scarlet fever. These changes are, however, in marked contrast to the Aschoff nodule in rheumatic fever. This nodule is as specific a granuloma as the lesions of tuberculosis or of Hodgkin's disease.

W. S. BOIKAN.

BONE MARROW IN THE SUPRARENAL GLANDS. J. SOÓS, Beitr. z. path. Anat. u. z. allg. Path. 85:611, 1930.

Soós reports the occurrence in otherwise normal suprarenal glands of pea-sized to walnut-sized circumscribed nodules of yellow, red and mixed bone marrow showing myelopoiesis and erythropoiesis. The yellow nodes have previously been called lipomas. The opinion is expressed that the nodes originate from microscopic foci of bone marrow.

W. S. BOIKAN.



**TELANGIOSTENOSIS—THE UNDERLYING PROCESS IN ENDARTERITIS OBLITERANS AND OTHER VASCULAR DISEASES.** S. KROMPECHER, Beitr. z. path. Anat. u. z. allg. path. **85**:646, 1930.

Telangiostenosis is a primary disease of the small blood vessels, arterial and venous, which leads to gangrene of the extremities and organic insufficiency. The stenosis is produced by intimal elastoblast proliferation and formation of elastic membranes. The elastoblast is the earliest cell to differentiate from the vascular mesenchyme. Thrombosis is always secondary. Intervascular increase of elastic elements occurs concurrently and is termed elastofibrosis. Telangiostenosis by itself or in association with atherosclerosis is considered the underlying process of endarteritis obliterans, arteritis obliterans, thrombo-angiitis obliterans, malignant nephrosclerosis and scleroderma.

W. S. BOIKAN.

**AORTIC LIPOID DEPOSITS IN CHILDREN.** A. SSOLOWJEW, Centralbl. f. allg. Path. u. path. Anat. **53**:145, 1931.

Ssolowjew believes the fatty specks found in the aortas of young children are the result of a milk diet high in cholesterol. The proof was furnished by the aortas of suckling guinea-pigs, which contained microscopic fat droplets in the interstitial tissue of the intima and inner portions of the media. Such deposits were increased by feeding pregnant guinea-pigs egg yolk and milk. Additional support is given to this theory by the finding of varying amounts of fat in the hepatic cells and hepatic duct cells of such sucklings, whereas fat is found only in the Kupffer cells of guinea-pigs on a diet of grains and vegetables. Egg yolk and milk apparently occasion the appearance of fat globules even within the intimal cells of the aorta and give rise to macroscopically demonstrable deposits.

GEORGE RUKSTINAT.

**ABNORMAL TISSUE FRIABILITY, HYPOFIBROSIS UNIVERSALIS.** C. BENEKE, Centralbl. f. allg. Path. u. path. Anat. **53**:177, 1931.

The patient was 49 years old at death and had a rather unusual history. At 18 years, she had had typhoid fever and thereafter remained well until she was 36. Then severe gastro-intestinal symptoms occurred, accompanied by vomiting and bowel movements as many as twenty-two per day. Such attacks occurred again ten, eleven and twelve years later. Thirteen years later colic, icterus and loss of weight led to operative treatment, and a gastro-enterostomy was performed with difficulty. During this operation, the serosa of the bowel and stomach became detached at the least touch, and a retractor applied to the liver caused a tear in it. Two days later death occurred from peritonitis and anemia. At autopsy the skin of the neck was almost entirely devoid of cutis; the fat of the body was normally abundant, except on the hands. The abdominal cavity contained about 500 cc. of free blood. The mesentery tore like spider-web; the abdominal muscles could be pulled away from the abdominal wall with very little force; the myocardium, aorta, liver and kidney were very friable. The spleen could be torn from the body easily; the duodenum burst when its removal was attempted. The friability of the tissues was due to the small amount of collagenic fibers.

GEORGE RUKSTINAT.

**PURULENT AORTITIS.** F. HAUBRANDT, Centralbl. f. allg. Path. u. path. Anat. **53**:327, 1932.

The classification of the 100 reported cases of purulent aortitis includes extension to the aorta from a neighboring infection, most commonly from an aortic endocarditis extending to the aortic intima or from a purulent process outside of the aorta extending to the adventitia. Less frequently the infection is carried in the blood of the aorta, and rarely by the vasa vasorum. Haubrandt reports a case of the commonest and then one of the rarest forms. The latter resulted from pneumococcic pneumonia of the right upper lobe. The cocci settled in the scars

of an old syphilitic aortitis and caused purulent aortitis. The etiologic factors were obvious; first pneumonia and then purulent aortitis, which was so extensive and protracted as to occasion purulent pericarditis. GEORGE RUKSTINAT.

AGRANULOCYTIC BLOOD PICTURE FOLLOWING TREATMENT WITH ARSPHENAMINE. CARL OESTEREICH, *Folia haemat.* **44**:137, 1931.

In a patient with tabes dorsalis there developed typical agranulocytosis following antisyphilitic treatment with a German arsphenamine. The case differed from those described by Schultz, in that there were no lesions of the mucous membranes and no icterus. The platelets, too, were diminished in number without, however, causing hemorrhages. It is certain that preparations of benzene and arsenic used in the treatment of syphilis are liable to induce a disease of the granulocytic apparatus.

B. M. FRIED.

SYPHILIS OF THE LUNG. F. LANDSBERG, *Virchows Arch. f. path. Anat.* **277**:583, 1930.

In the case presented, the clinical diagnosis of syphilis of the lung was based on the physical and roentgenologic findings and on the strongly positive serologic reactions. The man was 55 years of age and had contracted the syphilitic infection at the age of 20 years. Necropsy revealed, in addition to syphilitic meso-aortitis, marked scarring and contraction of the lung, with gummatous caseation and productive endarteritis. Spirochetes and tubercle bacilli could not be demonstrated by the appropriate staining methods.

W. SAPHIR.

LIPOID CELLULAR HYPERPLASIA IN LYMPHOGRANULOMATOSIS. H. FREIFELD, *Virchows Arch. f. path. Anat.* **277**:595, 1930.

In two cases of lymphogranulomatosis with characteristic histology, the reticulo-endothelial cells of the spleen and lymph nodes had proliferated and contained large quantities of lipoid material. The enlarged cells resembled somewhat those of Gaucher's disease.

W. SAPHIR.

THE PYELONEPHRITIC CONTRACTED KIDNEY. M. STAEMMLER and W. DOPHEIDE, *Virchows Arch. f. path. Anat.* **277**:713, 1930.

Staemmler and Dopheide review the literature and present cases of their own. The gross characteristics of the kidney contracted as the result of pyelonephritis are: variability of the alterations present in the two kidneys; diffuse distention of the renal pelvis and calices without apparent obstruction; irregularity and granulation of the surface of the kidney; irregular contraction of the parenchyma, and thickening of the mucosa of the pelvis, ureters and bladder. The histologic changes noted were: chronic inflammation of the pelvis and calices, with epithelial hyperplasia and metaplasia; chronic productive inflammation of the medulla, with scarring of the pyramids and connective tissue proliferation at the corticomedullary junction, and chronic inflammation of the cortex, leading to destruction of parenchyma and glomeruli and their replacement by scar tissue.

W. SAPHIR.

GIANT CELL PNEUMONIA IN AN ADULT. M. DUGGE, *Virchows Arch. f. path. Anat.* **277**:757, 1930.

This is the first recorded instance of giant cell pneumonia in an adult. The disease has been previously observed only in children. A farmer, 30 years of age, who had suffered for many years with asthma, died with symptoms of pneumonia. Necropsy revealed advanced chronic bronchopneumonia with bronchiolitis, chiefly of the upper lobes. There was extensive development of granulation and con-

nective tissue. Giant cells containing cholesterol crystals and concentric iron and calcium incrustation bodies were the most striking feature of the histologic picture. The giant cells were embedded in the granulation tissue and, according to Dugge were derived from the endothelium of the granulation tissue. The giant cells of the organizing pneumonia of children, he believes to be derived from the alveolar epithelium. Such alveolar epithelial giant cells were seen also in the case reported (See Du Bois, Franklin S.: *Chronic Bronchitis with Foreign Body [Elastic Fibers] Reactions in the Lungs*, ARCH. PATH. **12**:222, 1931).

W. SAPHIR.

## Pathologic Chemistry and Physics

IRON IN THE LIVER AND IN THE SPLEEN AFTER DESTRUCTION OF BLOOD AND AFTER TRANSFUSIONS. S. A. GLADSTONE, *Am. J. Dis. Child.* **44**:81, 1932.

Livers obtained at autopsies on fetuses and infants were studied microscopically and chemically to determine the variations in the amount of iron present and the factors on which the variations depend. There is no evidence microscopically or chemically of large or progressive depositions of iron in the liver during the last four months of intra-uterine life. Exclusive of iron as hemoglobin, the entire liver of the mature new-born infant contains on the average about 32 mg. of iron. The largest amounts of iron are found in the liver from one to ten weeks after birth, and these are believed to depend on postnatal intravascular destruction of blood. Hemosiderosis of the liver may also result from hemorrhages into the tissues or cavities of the body of the fetus or infant, and during fetal life it may result from similar hemorrhages in the mother, the liberated iron reaching the fetal liver via the placenta. Hemosiderosis of the spleen and the liver follows transfusions of blood, and the amount of iron found in the liver is influenced by the size and frequency of the transfusions. The appropriation and utilization of transfused blood are discussed. It appears that the changes occurring during the first three months of human life are comparable to the changes that occur during the first six days of postnatal life in the rabbit, namely, a doubling of the body weight, a moderate increase in the total hemoglobin, a marked decrease in the percentage of hemoglobin and an early loss of iron followed by a progressive increase of hemoglobin iron but more particularly of nonhemoglobin iron.

AUTHOR'S SUMMARY.

HISTOCHEMICAL STUDIES BY MICROINCINERATION OF NORMAL AND NEOPLASTIC TISSUES. G. H. SCOTT and E. S. HORNING, *Am. J. Path.* **8**:329, 1932.

The results obtained from this investigation are of interest, as they have demonstrated that functional differences between cancer and normal tissues are exhibited inorganically by marked variations in their inorganic content. An additional feature is the close similarity between developing embryonic cells and cancer cells—a similarity which is mainly due to the distribution and arrangement of mineral salts. Both of these cells are characterized by an extraordinary variation in the intensity, concentration and orientation of their inorganic constituents, and contrast greatly, on the other hand, with healthy adult tissue in the appearance of the mineral elements, which in the latter remain proportionally fixed. This "inorganic reversion" of the cancer cell, as revealed by micro-incineration, is interesting in view of Cohnheim's theory to the effect that malignancy depends on the retention of small groups of cells of embryonal character.

AUTHORS' SUMMARY.

CHOLESTEROL CONTENT OF BLOOD IN EPILEPSY AND IN FEEBLEMINDEDNESS. H. GRAY and L. C. MCGEE, *Arch. Neurol. & Psychiat.* **28**:357, 1932.

One hundred and eight samples of blood from feeble-minded patients over 20 years of age and 623 samples from that of epileptic patients over 20 years of age,

taken at various intervals after meals, have been studied by Bloor's method (without saponification) for cholesterol content, in contrast with blood from normal persons. It was found that cholesterol is sufficiently stable to permit the use of samples of oxalate plasma that has stood four days or of whole blood that has stood a week or longer (three weeks). The adolescent persons with epilepsy had a slightly lower level of cholesterol than the adults. Within one hour after an epileptic seizure the cholesterol average reached a low point. It was somewhat higher at intervals of from two hours to one week than during the first hour, and again it was at a lower level at intervals of from one to four weeks. At intervals of more than one month after seizures, the cholesterol average rose to 172 mg. (in normal contrasts it was 190 mg.). The influence of meals was found to be negligible. The average cholesterol value of the whole blood was found to be 194 mg. per hundred cubic centimeters for normal men, 165 mg. for persons with epilepsy and 154 mg. for feeble-minded persons. After convulsions, there is a drop in cholesterol, followed by a gradual rise which continues for a month though it does not reach the average value for normal persons, the difference being about 10 mg. Near the attack the ratio is higher. In other words, the cholesterol in the blood of persons with epilepsy is low, and in feeble-minded persons it is even lower. The high fat diet recommended in the treatment of epilepsy is thus justified also on theoretical ground.

GEORGE B. HASSIN.

ESTIMATION OF PROTEINS BY THE PRECIPITIN REACTION. G. L. TAYLOR, G. S. ADAIR and M. E. ADAIR, *J. Hyg.* **32**:340, 1932.

The amount of crystallizable albumin in egg-white and the amount of total globulin in horse serum have been estimated by means of the precipitation reaction. The results are in good agreement with those obtained by other methods. For such estimations it appears advisable to use only antisera prepared against individual proteins.

AUTHORS' SUMMARY.

CAUSES OF CELL DEATH IN IRRADIATED HUMAN TISSUE. B. D. PULLINGER, *J. Path. & Bact.* **35**:527, 1932.

In living vascular tissues, as opposed to in vitro preparations and young embryonic cells, hyperemia is an essential reaction to therapeutic irradiation with radium and x-rays. Thin-walled, loosely supported capillaries and veins are most readily affected and react in deep structure as well as at surfaces. If endothelial injury follows excessive distention, hyperemia is succeeded by exudation of serum, extravasation of blood and intravascular thrombosis. All effects following irradiation are related to these two phases, namely, vascular stimulation and vascular degeneration. Problems which remain to be solved are concerned with the particular kind of radiation which starts the reaction and with the immediate hyperemic stimulus. Is the former beta or gamma? Is the latter physical or due to liberation of a chemical product such as histamine?

AUTHOR'S SUMMARY.

BILE PRECIPITATION AND BILIARY CALCULI. C. E. NEWMAN, *Beitr. z. path. Anat. u. z. allg. Path.* **86**:187, 1931.

In human bile, the ratio of bile acids to cholesterol is within certain limits a constant one. Hepatic injury may disturb this ratio in favor of relatively increased cholesterol and thus favor the formation of calculus. The bile from gallbladders containing calcium pigment cholesterol stones shows a relatively decreased bile acid content with consequent decreased ability to keep the cholesterol in solution. Bile in pigment calculus shows a normal ratio of constituents. Sediments are found in practically all bile and more often in normal than in abnormal gallbladders. Neither these nor the so-called microlithia (spheroid bodies from 10 to 30 microns in diameter) have any relation to the formation of biliary stone.

W. S. BOIKAN.

URIC ACID CONTENT OF BLOOD FROM THE UMBILICAL CORD. IWAN MANOLOFF, Frankfurt. *Ztschr. f. Path.* **42**:188, 1931.

Determinations were made in 120 cases. In 66, the uric acid content was less, and in 54 it was more than 4 mg. per hundred cubic centimeters. In eleven children, the uric acid content amounted to from 5.5 to 8.52 mg. per hundred cubic centimeters. The author believes that the average uric acid content in the newborn is higher the longer the labor lasts. In children of primiparae the uric acid content, as a rule, is higher than 4 mg. per hundred cubic centimeters.

O. SAPHIR.

LIPOLYTIC ACTIVITY OF BLOOD SERUM AND CEREBROSPINAL FLUID AFTER DEATH. E. BACH and L. LUSZTIG, *Virchows Arch. f. path. Anat.* **280**:325, 1931.

In preliminary experiments the lipolytic activity of the blood serum was determined one or two days before death and again after death; the changes noted fell within the limits of error of the method used, which was that of the splitting of tributyrin. Repeated examinations up to twenty-four hours after death also showed no change in lipase content. In general, the lipolytic activity of the serum bore a direct relation to the nutritional condition, being high in bodies with much fat and low in those with little adipose tissue. In cachexia and uremia, the lipase content of the serum was decreased; in acute febrile infectious diseases it was increased. In early tuberculosis it was normal or only slightly decreased, whereas in late tuberculosis with caseation it was greatly decreased or absent. The lipolytic activity of the cerebrospinal fluid was less than that of the blood serum, but in general rose or fell with the latter. The authors suggest that postmortem studies of lipase may be of value in the study of pathologic fatty change, intoxications, and a variety of diseases.

O. T. SCHULTZ.

LEAD CONTENT OF HUMAN BONE. E. BARTH, *Virchows Arch. f. path. Anat.* **281**:146, 1931.

The P. Schmidt micromethod was used for the determination of the lead content of bones of persons of various ages who had never had lead poisoning or close contact with lead in industry. The bones of infants contained 0.01 to 0.03 mg., those of young adults from 0.03 to 0.05 mg., and those of older adults from 0.08 to 0.14 mg. The same slight progressive increase was noted in the inhabitants of villages without a central water supply as in cities. The author believes that the lead is derived from foods that normally contain small quantities of lead.

O. T. SCHULTZ.

CALCIUM CONTENT OF THE ARTERIES OF THE UTERUS. W. ZINKANT, *Virchows Arch. f. path. Anat.* **281**:911, 1931.

Quantitative chemical methods yield accurate information respecting the content of the estimated substance in a tissue, but give little information respecting the distribution of the substance in relation to the tissue elements. Histochemical and histologic methods may permit the detection of certain substances and may make it possible to study the distribution of the substances, but such methods may fail to detect smaller quantities of substance that the chemical method reveals. The Schultz-Brauns method of ashing frozen sections of fresh, unfixed tissues is said to give more accurate information on the presence and localization of certain substances, especially calcium and iron, than other methods. Zinkant applied this method to a study of the calcium content of the arteries of the uterus. By this method calcium was found to be present in the second and third decades of life, at a time when the usual histochemical methods fail to reveal its presence. The

element is present in both media and intima, and increases progressively with age. During the fifth decade, when the usual methods may reveal the presence of large masses of calcium in the media, the Schultz-Brauns method demonstrates the presence of calcium in the smaller arteries and even in the arterioles. No relation between the calcium content of the uterine arteries and the number of previous pregnancies could be detected.

O. T. SCHULTZ.

GALACTOSIDES AND LIPOID METABOLISM. P. KIMMELSTIEL, *Virchows Arch. f. path. Anat.* **282**:402, 1931.

In studies of lipid metabolism and of the lipid content of pathologic tissues, most attention has been paid to cholesterol and its esters. In some of the pathologic states characterized by lipid infiltration, the phosphatides and cerebroside have received attention, but not as much as their importance in general lipid metabolism and in the pathology of the lipoids warrants. The object of the studies reported by Kimmelstiel has been the lipoids characterized by the presence of a galactose molecule, the galactosides, which have been more often referred to in pathologic literature as cerebroside. The cholesterol, phosphatide and galactoside fractions of the lipid complex were determined quantitatively in the human aorta at different ages. The cholesterol fraction was largest, the phosphatide next, and the galactoside third. The three fractions increased *pari passu* with the degree of lipid infiltration evident to the naked eye. Cholesterol increased disproportionately and markedly when atheromatous degeneration was evident. The high cholesterol content does not appear until degeneration and death of tissue have occurred, with disturbance in the interrelations of the elements of the lipid complex. In acute feeding experiments, in which pure cholesterol in sesame oil was fed to young rabbits, the phosphatide and galactoside content of the liver increased 89 and 83 per cent, respectively; cholesterol, only 50 per cent. In the kidney there was no appreciable increase of cholesterol, but phosphatide and galactoside increased 43 and 53 per cent, respectively. Comparative physicochemical studies of different lipoids revealed that the galactosides have an intermediate position in the series of hydrophobe and hydrophil colloids. The physicochemical properties of the galactosides are an indication of their importance in lipid metabolism. In atherosclerosis, cholesterol does not have the significance that has been attached to it, since the increase that has been considered significant does not occur until tissue has died. Previous to the stage of local tissue death, the phosphatides and galactosides increase proportionately as much as does cholesterol.

O. T. SCHULTZ.

THE LIPOID CHEMISTRY OF XANTHOGRAULOMATOSIS. H. KLEINMANN, *Virchows Arch. f. path. Anat.* **282**:613, 1931.

Kleinmann made quantitative determinations of the lipoids of tissues from the case of xanthogranulomatosis reported by Ighenti (*Virchows Arch. f. path. Anat.* **282**:585, 1931). His results are in general agreement with those of Chiari. The essential findings were: an increase in total lipoids due to a relative and absolute increase in cholesterol and its esters, a reversal of the ratio of cholesterol ester to cholesterol as compared with normal tissues, and a reversal of the ratio of cholesterol to lecithin as compared with Niemann-Pick's disease. The ratio of cholesterol to lecithin was 5.85:1, as compared with 1:9.3 for the spleen of Niemann-Pick's disease. The ratio of cholesterol ester to cholesterol was 5.08:1, as compared with from 1:2 to 1:4 for normal tissues. The total lipoids of the spleen were not increased, but the ratio of cholesterol to lecithin was altered, being 2.7:1. For the normal spleen this ratio is 1:1.8, and for the spleen of Niemann-Pick's disease, 1:9.3. The ratio of cholesterol ester to cholesterol was 3.5:1. The total cholesterol of the liver was increased, being 3.86 per cent as compared with 1.28 per cent for the normal liver. The ratio of cholesterol to lecithin was 3.7:1.

O. T. SCHULTZ.

HISTOCHEMICAL STUDY OF URIC ACID INFARCTS OF THE KIDNEY OF THE NEW-BORN INFANT. SABINE EHRLICH, *Virchows Arch. f. path. Anat.* **283**:194, 1932.

The material that forms the so-called uric acid infarcts of the kidney of the new-born infant consists of rounded, concentrically laminated and radially striated concretions in the lumens of the straight tubules. Small numbers of similar concretions are found also in the tubules of the cortex. Microchemical methods demonstrated that the material is composed chiefly of uric acid and sodium, to which calcium, phosphoric acid and oxalic acid are added. O. T. SCHULTZ.

AMOUNT AND ARRANGEMENT OF THE BROWN PIGMENT OF HEART MUSCLE. O. OTTO, *Virchows Arch. f. path. Anat.* **283**:611, 1932.

Brown pigment is present in normal cardiac muscle at the latest by the twentieth year of age. It is situated at either one or both poles of the nucleus. In the actively functioning hypertrophied myocardium, the formation of pigment proceeds more slowly than in normal muscle. If the mass of pigment is situated at one pole of the nucleus in the normal cell, it becomes bipolar when the muscle hypertrophies. When the action of the hypertrophied muscle becomes weakened or insufficient, pigment deposition is increased, the new-formed pigment being at first localized at one pole of the nucleus. O. T. SCHULTZ.

IRON CONTENT OF THE ARTERIOSCLEROTIC AORTA. I. H. PAGE and W. MENSCHICK, *Virchows Arch. f. path. Anat.* **283**:627, 1932.

According to older histochemical investigations, the iron content of bone is temporarily increased during physiologic calcification. Twenty-four aortas of persons from 19 to 82 years old were analyzed quantitatively for their total content of iron. Three of the aortas were normal; the rest exhibited varying degrees of degeneration and calcification. Organic phosphorus was also determined. The iron content was not increased, and there was no parallelism between the degree of calcification and the quantity of total iron or that of loosely bound iron present. Organic phosphorus increased with the degree of calcification. O. T. SCHULTZ.

RESPIRATORY MECHANICS OF THE LUNG. O. THIES, *Virchows Arch. f. path. Anat.* **284**:772 and 796, 1932.

The first communication is a critical review of the literature dealing with the measurement of mechanical factors operative in the lungs during respiration. The second is an account of the author's attempts to evaluate some of these factors in the lung after death by means of the Gildemeister elastometer, which measures the elasticity of a tissue in terms of changes in hardness. The work reported represents a considerable amount of labor and an ingenious technic, but it resulted in no very definite conclusions. O. T. SCHULTZ.

CHEMICAL CHANGES IN PARENCHYMATOUS DEGENERATION. V. UHER, *Virchows Arch. f. path. Anat.* **284**:880, 1932.

The potassium content of the liver in a state of cloudy swelling is relatively increased; the sodium content, relatively decreased. This disturbance in the relation of potassium and sodium ions leads to increased retention of water by the cell, to increased dispersion of the plasma colloids, and to maximum swelling of the colloids. O. T. SCHULTZ.

BENCE-JONES PROTEIN. OTTO JERVELL, Norsk mag. f. lægevidensk. **93**:622, 1932.

In a case of multiple myeloma, Bence-Jones protein was found in the blood and urine. Spontaneous crystallization of the protein took place when the urine was allowed to stand for some time. The crystals were easily dissolved in alkaline solution; on adding acetic acid, precipitation occurred again, with the reformation of crystals in a short time. The crystals formed of finer and larger needles as well as spheres and boat shapes. Crystals recrystallized three times and dried in the air were employed for determining the iso-electric point, which was found to lie between  $p_H$  4 and  $p_H$  4.25. The serum coagulated on heating to 56 C. The total quantity of protein in the serum was 9.08 per cent with 5.09 per cent globulin and 3.99 per cent albumin. On mixing 0.5 cc. of serum with 4.5 cc. of salt solution and two drops of 2 per cent acetic acid, a white precipitate formed after two or three hours at 58 C. Control serum treated in this way gave no precipitate. The Bence-Jones protein was obtained by heating the diluted and acidulated serum to the boiling point and filtering over a boiling water bath; from the filtrate the protein was salted out with ammonium sulphate.

### Microbiology and Parasitology

BRAIN TO BRAIN TRANSMISSION OF THE SUBMAXILLARY GLAND VIRUS IN YOUNG GUINEA PIGS. N. P. HUDSON and F. S. MARKHAM, J. Exper. Med. **55**: 405, 1932.

The virus of the submaxillary glands of guinea-pigs was transmitted serially from brain to brain in young guinea-pigs. Successful transmission was shown by nervous symptoms, death and typical meningo-encephalitis. Increase in virulence of the virus or adaptation to the brain tissue was not observed. Fifteen days or more after cerebral inoculation, typical cellular inclusions were found in the salivary gland.

THE ASSOCIATION OF PNEUMOCOCCI, HEMOPHILUS INFLUENZAE, AND STREPTOCOCCUS HEMOLYTICUS WITH CORYZA, PHARYNGITIS, AND SINUSITIS IN MAN. L. T. WEBSTER and A. D. CLOW, J. Exper. Med. **55**:445, 1932.

Pneumococci, *H. influenzae* and *S. hemolyticus* are known to be frequent inhabitants of the upper respiratory tract, but most workers have not recognized any definite relationships between their presence and coryza, sore throat, influenza and sinusitis. Dochez, Shibley and Mills, however, in experimental studies of common cold, stated that in both spontaneous and experimentally induced "colds" in anthropoid apes, the "most significant change observed has been the increase of activity on the part of the potential pathogens habitually present in the throat flora. Coincident with the appearance of symptoms, pneumococci, *S. hemolyticus*, and *B. Pfeifferi* have developed in greatly increased numbers and have spread over a wide area of the nasopharyngeal mucous membranes. These organisms became at this time conspicuous even in the nose, where they are seldom or never present under normal conditions. The same phenomena have not been observed in human beings." The essential facts of the present observations are that persons free from pneumococci, *H. influenzae* and *S. hemolyticus* are in general free from coryza, sore throat, influenza and sinusitis; that persons who are occasional or periodic carriers of these organisms may not show the presence of the organisms in tests over long periods of health, but generally yield positive cultures during or following attacks and subsequently again give negative results in tests; finally, that persons who are chronic carriers show, during these illnesses, increasing numbers of organisms in the throat and extension of the organisms to the nose. That these organisms may be the actual incitants has been claimed by Park. That they are secondary invaders is the view of Shibley, Mills and Dochez, who stated as a result of their experimental work on this subject that "the most important



significance of viruses of this type (common cold) seems to lie in their capacity to incite activity on the part of the more dangerous pathogenic organisms that infect the upper respiratory tract." The present observations bring out the intimate relationship between these pathogens and symptoms of disease of the upper respiratory tract, but do not disclose the nature of the relationship. Finally, an addition has been made to the knowledge of the mode of spread of these organisms. A focus of growth and dissemination has been determined in the nasal passages and throat of the person who suffers from chronic disease of the upper respiratory tract, and increases in numbers of the organisms at the focus and their spread to contacts have been related to the winter season and to the occurrence of symptoms in the carrier. The observations suggest that the dosage of these organisms in a community is controlled by the resistance of the carrier and of the contacts. This view is an agreement with the facts derived from studies of native animal infections.

#### AUTHORS' SUMMARY.

THE AGENT OF FOWL LEUCOSIS. J. FURTH and H. K. MILLER, J. Exper. Med. **55**:465, 479 and 495, 1932.

*Concentration in Blood Cells and Plasma (J. Furth).*—The concentration of the transmitting agent of leukosis in fowls, as determined by titration, was approximately the same in suspensions of blood cells and in cell-free plasma; the smallest amount of plasma producing leukosis was 0.000001 cc., and of cell suspension, 0.00001 cc. This observation excludes the possibility that transmission of leukosis by plasma is due to the presence of a small number of leukemic cells in the plasma. The success of inoculations with plasma (20 to 28 per cent of fowls) was, within wide limits, independent of the amount injected ( $10^{-1}$  to  $10^{-6}$  cc.). The percentage of successful inoculations with varying quantities of plasma was lower than with corresponding amounts of suspensions of cells (from 33 to 71 per cent). When plasma containing the transmitting agent was injected in decreasing amounts, the period of incubation of the leukosis was conspicuously lengthened. With decreasing amounts of a suspension of leukemic cells, the period of incubation was not so frequently or so greatly prolonged.

*Filtration of Leukemic Plasma (J. Furth and H. K. Miller).*—The agent transmitting leukosis readily passed all types of silicious filters. Filtration was particularly successful when the plasma was freed from particles and substances that would otherwise obstruct the pores of the filter. Filtration through fine filters seemed to be facilitated by preceding filtration through coarse filters. A comparison of the periods of incubation of leukosis produced by unfiltered plasma and plasma passed through silicious filters showed that as a result of filtration, the period of incubation was somewhat prolonged. This suggests a slight or moderate decrease in the concentration of the transmitting agent in the plasma caused by filtration. Filtration through collodion membranes indicated that the agent transmitting leukosis is much smaller than the virus of bovine pleuropneumonia (250 microns), and that it approximates the size of bacteriophage.

*Resistance to Desiccation and Other Factors (J. Furth).*—The filtrable agent transmitting leukosis resisted drying, retaining its activity for at least fifty-four days. The conditions of successful desiccation have not been precisely ascertained. By the addition of glycerin, the agent could be preserved for at least one hundred and four days. It was not inactivated by freezing in liquid air. At 37.5 C., it lost its activity within fourteen days, but retained some of its activity for at least fourteen days when kept at 4 C.

#### AUTHORS' SUMMARIES.

THE CULTIVATION OF THE TYPHUS FEVER RICKETTSIA. C. NIGG and K. LANDSTEINER, J. Exper. Med. **55**:563, 1932.

*Rickettsia prowazeki* can be cultivated for many generations in vitro, without diminution in numbers or in virulence, in mediums similar to those described by

Maitland, Rivers and others for the cultivation of certain viruses. In all probability, such cultures can be maintained indefinitely. It has been impossible, thus far, to cultivate the rickettsia of typhus without employing living tissue.

AUTHORS' SUMMARY.

THE RELATIONSHIP OF PATHOGENIC BACTERIA TO UPPER RESPIRATORY DISEASE IN INFANTS. Y. KNEELAND, JR., and C. F. DAWES, J. Exper. Med. **55**:735, 1932.

Bacteriologic and clinical observations on respiratory disease in a semi-isolated infant population over a period of two years are recorded. In two severe winter outbreaks of respiratory infection, a parallel rise in the percentage of carriers of pathogenic organisms was noted. The first autumnal outbreak of colds seems to favor dissemination of the pathogenic organisms. The relationship of colds to the severer infections is roughly reciprocal. Infants between 8 and 14 months of age are subject to the most severe infections. The number of infants showing positive cutaneous reactions to products of pathogenic organisms increases during the winter months. The significance of these findings is discussed.

AUTHORS' SUMMARY.

THE RÔLE OF INTRACELLULAR BACTERIOPHAGE IN LYSIS OF SUSCEPTIBLE STAPHYLOCCCI. J. H. NORTHROP and A. P. KRUEGER, J. Gen. Physiol. **15**: 329, 1932.

The experiments described show that the significant condition for the occurrence of lysis is either a concentration of about 110 phage units inside each bacterium or of about  $12 \times 10^8$  units in each millimeter of surrounding solution. Since these two quantities are always in constant ratio to each other, it is immaterial which one is used. Evidently, also, they cannot be distinguished by any experimental procedure carried out under equilibrium conditions. It is perhaps more reasonable to suppose that the internal phage is responsible for the reaction.

AUTHORS' SUMMARY.

VARIANTS OF BACTERIUM PARADYSENTERIAE AND BACTERIUM MORGANI. G. M. MACKENZIE and L. N. BATT, J. Immunol. **22**:257, 1932.

From a culture of *B. paradysenteriae* isolated during an epidemic two stable variants have been produced by growth in homologous immune serum broth. In colonial structure, sugar fermentations and agglutinative, agglutinogenic and agglutinin-absorption properties, the variants have shown distinct and persistent differences from the original culture. One variant, although forming granular emulsions, is inagglutinable both in homologous and in heterologous immune rabbit serum, nor does it absorb agglutinin from antiserum produced with the culture from which it was dissociated. The other variant has been shown to have lost an antigenic component present in the original culture, and to have acquired an antigenic component not present in the original culture. This variant has acquired an agglutinative component common to it and a culture of *B. morgani*. This culture of *B. morgani* has an agglutinative and agglutinin-absorbing component common to it and the paradysentery strain. These two components, one common to cultures of *B. paradysenteriae* and *B. morgani* and the other common to cultures of the variant and *B. morgani* appear to be different.

AUTHORS' SUMMARY.

THE ENCEPHALITOGENIC POWER OF VACCINIA VIRUS. R. THOMPSON and L. BUCHBINDER, J. Immunol. **22**: 267, 1932.

Presence or absence of the encephalitogenic property in various strains of the virus of vaccinia cannot be ascribed to heterogenetic origin, since derivants of the same strain differ in this respect. Variations in the character of the cutaneous

lesions produced by neurotropic and dermatropic viruses are apparently associated with presence or absence of the ability to produce encephalitis. Encephalitogenic strains are not obviously contaminated with herpes virus, since recovery from neurovaccinial infection affords as little immunity to herpes virus as does recovery from dermovaccinial infection. The power of neurotropic strains to produce encephalitis is not referable to contamination with the virus of poliomyelitis, since the latter virus does not cause a nonencephalitogenic strain of the virus of vaccinia to become encephalitogenic for rabbits. Evidence is presented which indicates that neurotropic strains are not contaminated by some unknown virus capable of producing encephalitis alone or when aided by vaccinial infection. The encephalitis-producing power of certain of the strains is probably a property of the virus. It is suggested that the presence or the absence of this property may be due to a process similar to that of dissociation in bacterial cultures.

AUTHORS' SUMMARY.

BACTERIAL ENDOTOXIN (IN *SALMONELLA PULLORUM*). J. H. HANKS and L. F. RETTGER, *J. Immunol.* **22**:283, 1932.

The cell bodies of cultures of *S. pullorum* contain, and by appropriate methods of extraction yield, a relatively heat-resistant poison which is highly toxic for rabbits and capable of killing guinea-pigs and mice. This toxin did not cause loss of weight or other noticeable symptoms of illness in chicks, regardless of the route by which it was introduced. The toxic principle was fairly stable in hydrogen ion concentrations ranging from  $pH$  3 to 12, and did not deteriorate during exposure to direct sunlight for twenty-four hours. It was destroyed by prolonged action of trypsin or of pepsin. It was not dialyzable through parchment bags, and could be precipitated with ammonium sulphate or acetic acid alcohol. When the toxin of *S. pullorum* was introduced into the skin of normal rabbits, the severity of the reaction was correlated with the toxicity of the same preparation for mice. Repeated injections of culture filtrate or of cellular antigen into rabbits gave rise to specific agglutinative antibodies and to nonspecific cutaneous hypersensitiveness. Immunization with toxin filtrate induced tolerance to the toxin, but did not afford protection against subsequent infection with live culture. It was impossible to demonstrate that growth or the production of toxin is materially increased under tensions of carbon dioxide and oxygen that approximate those of animal tissues. The disease caused by *S. pullorum* appears to be a septicemia, rather than a toxemia.

AUTHORS' SUMMARY.

## Immunology

QUANTITATIVE STUDIES ON THE PRECIPITIN REACTION. M. HEIDELBERGER and F. E. KENDALL, *J. Exper. Med.* **55**:555, 1932.

A method, based on the precipitin reaction, is given for the microdetermination of the specific polysaccharide of the type III pneumococcus. As little as 0.01 mg. can be determined. The method should be applicable to any specific polysaccharide on standardization of a homologous antibody solution or antiserum in the region of excess antibody.

AUTHORS' SUMMARY.

REACTION OF RABBITS TO GREEN STREPTOCOCCI. M. P. SCHULTZ and H. F. SWIFT, *J. Exper. Med.* **55**:591, 1932.

Rabbits were rendered very hypersensitive by relatively small doses of green streptococci given intracutaneously, and somewhat less hypersensitive by similar doses of heat-killed vaccine prepared from hemolytic streptococci. Animals receiving the same doses intravenously gave, on subsequent testing, lesions slightly more marked than normal controls; but these lesions were qualitatively hard and nodular compared with the large, edematous lesions in the cutaneously sensitized group.

There was no parallelism between the degree of cutaneous or ophthalmic hypersensitivity and the agglutinin titer of the blood serum. Hypersensitivity to whole streptococci appears to depend more on previously induced focal infection than on circulating antibodies.

AUTHORS' SUMMARY.

SEROLOGY OF SYPHILIS: THE POSITIVE WASSERMANN REACTION IN NORMAL RABBITS. H. EAGLE, J. Exper. Med. **55**:667, 1932.

More than one half of normal rabbits contain complement-fixing or precipitating antibodies against Wassermann antigens (the alcohol-soluble lipoids of beef, rabbit and human hearts) by a sufficiently sensitive technic. Normal human serums tested by the same technic are uniformly negative. Intravenous injection of colloidal suspensions of the lipoids of beef and human hearts into rabbits occasionally causes a significant increase in the titer of this normal Wassermann (antilipoid) reaction. This may indicate a certain degree of antibody response to the lipoids as such; it may be due to the presence in such extracts of traces of foreign protein, which would activate the lipid hapten into a complete antigen; or it may be a nonspecific increase in a normal antibody, not due to a specific antigenic stimulus. Confirming the results of Sachs, Klopstock and Weil, the addition of normal foreign (human) serum to the lipoids of rabbit, beef and human hearts makes them antigenic for rabbits. Intravenous injection of such lipid-serum mixtures usually causes a significant increase in the titer of the complement-fixing and precipitating antibody against tissue lipoids. The precipitate that forms on the addition of tissue lipoids to human syphilitic serum is by far the most efficient antigen for the production, in rabbits, of antibodies to tissue lipoids that I have as yet encountered. Rabbits treated by intravenous injection of such a precipitate regularly present a Wassermann reaction the titer of which is many times higher than either that observed in human syphilis or that induced by the injection of a mixture of normal serum and lipid. The marked antigenic property of the precipitate as compared with that of a mixture of normal serum and lipid is considered to be due to the fact that it contains a foreign protein firmly bound to the lipid particles, namely, the human reagin-globulin with which they have combined. This interpretation is supported by the observations that heating at 100 C., which does not affect the lipid constituent of the precipitate, destroys its antigenic power for rabbits, and that a similar precipitate derived from Wassermann-positive rabbit serum instead of syphilitic human serum, and therefore containing tissue lipid in combination with homologous (rabbit) protein, is completely nonantigenic for rabbits.

AUTHOR'S SUMMARY.

CHEMOIMMUNOLOGICAL STUDIES ON CONJUGATED CARBOHYDRATE-PROTEINS. O. T. AVERY, W. F. GOEBEL and F. H. BABERS, J. Exper. Med. **55**:761 and 769, 1932.

The synthesis of  $p$ -aminophenol  $\alpha$ -glucoside has been described. This glucoside can be coupled to any protein to yield a synthetic  $\alpha$ -glucoside-protein complex. A synthetic  $\beta$ -glucoside-protein complex has also been prepared. These synthetic sugar-protein complexes have been used as immunizing antigens in order to ascertain whether  $\alpha$ -glucosidic and  $\beta$ -glucosidic unions influence the specificity of the immune response in animals.

In the case of the synthetic antigens containing the A and B compounds of dextrose (glucose) alone, the evidence indicates that the immunologic relationships of the reactive glucosides are determined by known variations in their chemical constitution and are independent of the protein to which they are attached. In view of these findings it seems not unlikely that in the case of the polysaccharides, because of their more complicated structure and the greater possibility for variation in molecular configuration, there may be found many examples of a similar overlapping specificity among carbohydrates of unrelated origin.

AUTHORS' SUMMARIES.

THE SEROLOGICAL SPECIFICITY OF PEPTIDES. K. LANDSTEINER and J. VAN DER SCHEER, *J. Exper. Med.* **55**:781, 1932.

With the idea that studies on the serologic properties of peptides may ultimately aid in the understanding of the precipitin reactions of proteins, antigens have been prepared containing aminobenzoylated dipeptides, namely glycylglycine, glycyl-d-l-leucine, d-l-leucyl-glycine and d-l-leucyl-d-l-leucine. These four antigens were found to be different serologically, their specificity depending on the structure of the terminal amino-acid carrying the free carboxyl group, and to a less degree also on the second amino-acid. The results were obtained by means of precipitin and inhibition tests. Analogies to observations on the specificity of enzymes are discussed.

AUTHORS' SUMMARY.

THE RELATION OF HYPERSENSITIVENESS TO LESIONS IN THE LUNGS OF RABBITS INFECTED WITH PNEUMOCOCCI. L. A. JULIANELLE and C. P. RHOADS, *J. Exper. Med.* **55**:797, 1932.

Intratracheal injection of egg albumin or pneumococcic protein into rabbits previously inoculated with the respective antigen induces an inflammatory reaction in the lungs. A similar reaction occurs following intratracheal injection of pneumococcic protein into rabbits previously inoculated with heat-killed suspensions of the bacteria. This reaction appears to be related to the presence of circulating antibody and to have the nature of the Arthus reaction. A study of the reactions of the lungs of rabbits to infection caused by intravenous injections of pneumococci reveals that reactions occur irregularly in the lungs; in the lungs in which they occur, the histologic changes are not different as between normal rabbits and rabbits made resistant by previous intravenous or intracutaneous injections of pneumococci. Intratracheal injection of pneumococcic protein followed by intravenous injection of virulent pneumococci on the next day does not alter the course and character of the infection in resistant rabbits. The experiments reported in this paper bring no evidence to support the view that the lesions in the lungs of rabbits following intravenous injection of pneumococci are modified by any previous state of sensitivity.

AUTHORS' SUMMARY.

ANTIBODIES AGAINST VACCINIA VIRUS. R. THOMPSON, E. L. HAZEN and L. BUCHBINDER, *J. Immunol.* **22**:189, 1932.

Serums from rabbits hyperimmunized by intravenous and intraperitoneal injections of tissues (brain, testicle and skin) containing vaccinia virus caused fixation of alexin in the presence of a suitable dilution of vaccinia brain or skin suspension, but not in the presence of nonvaccinia tissue suspensions. The evidence indicates that this fixation is a process specific for tissue containing vaccinia virus, regardless of the tissue in which the virus is propagated, and that it is not due to the immune reactions of concomitant bacteria.

AUTHORS' SUMMARY.

NATURAL AGGLUTININS AND THEIR RELATIONSHIP TO THE SOMATIC AND FLAGELLAR ANTIGENS OF BACTERIA. H. J. GIBSON, *J. Immunol.* **22**:211, 1932.

Normal serum from various mammalian animals contains agglutinins that react with the H and O antigenic constituents of many bacteria. Flagellar suspensions have been used to demonstrate H-agglutinins. Agglutinin-absorption experiments show that the specificity of natural agglutinins (as described in a previous communication) depends chiefly on the H type. The O type appears to possess affinities for antigenic constituents that are more widely shared by different organisms. It was not found possible to demonstrate the antigenic relationship among members of the *Salmonella* and *Bacillus proteus* X groups so precisely with normal serums as with immune serums. The thermolability of the O type of agglutinins was found to be greater than that of the H type in the normal serum of a number

of animal species. Both showed greater lability than the corresponding immune agglutinins. Rough and smooth variants of the same bacterial strain showed antigenic differences in their reactions with normal serums.

AUTHOR'S SUMMARY.

ELECTRIC CHARGE OF BACTERIAL ANTIGENS. L. OLITZKI, J. Immunol. **22**:251, 1932.

The H-antigen of *B. proteus* X19 carries a negative electric charge over a  $p_H$  range of from 12 to 4.4; the O-antigen, over a range of from 12 to 3.4. By cataphoresis of whole bacteria at  $p_H$  4, it is possible to obtain large amounts of pure O-antigen at the positive pole of the apparatus. By cataphoresis of bacterial extracts at  $p_H$  4, it is possible to remove the O-antigen completely, leaving pure H-antigen in the middle vessel of the apparatus.

AUTHOR'S SUMMARY.

SKIN REACTIONS TO HUMAN AND AVIAN TUBERCULIN IN DISEASES OF LYMPHOID AND MYELOID TISSUE. F. PARKER, JR., H. JACKSON, JR., G. FITZ HUGH and T. D. SPIES, J. Immunol. **22**:277, 1932.

Tuberculin tests with both human and avian tuberculin were carried out on patients with Hodgkin's disease, malignant lymphoma, leukemia, pernicious anemia and cancer. Similar tests were done on tuberculous and normal persons. Fewer positive reactions were obtained in the patients with diseases of the lymphoid and myeloid tissues and those with malignant disease than in normal persons and tuberculous patients. More positive reactions to avian than to human tuberculin were found, except in the normal group.

AUTHORS' SUMMARY.

PROGRESSIVE, SELECTIVE ABSORPTION OF PRECIPITINS IN MULTIVALENT SERUM. L. HEKTOEN and E. DELVÉS, J. Infect. Dis. **50**:237, 1932.

Precipitins in multivalent serum may be removed successively with reasonable success by the selective action of single antigens.

AUTHORS' SUMMARY.

FLOCCULATION TESTS FOR THE DIFFERENTIAL DIAGNOSIS OF SMALLPOX AND CHICKENPOX. L. C. HAVENS and C. R. MAYFIELD, J. Infect. Dis. **50**:242, 1932.

Flocculation tests with thirty-eight specimens of serum in thirty-five cases of smallpox gave positive results with all but five. Four of the negative specimens were taken during the first week of the disease. Two cases in which successive specimens were obtained showed an increase in titer during the course of the disease. Eight specimens of serum in seven cases of chickenpox gave negative results. Flocculation tests with smallpox scabs and immune rabbit serum gave definitely positive results with dilutions of the antigens as high as 1:2,000. Chickenpox scabs invariably failed to flocculate in dilutions higher than 1:500. The flocculation obtained with lower dilutions of the chickenpox antigens occurred also with normal rabbit serum, and its nonspecific character was further demonstrated by absorption of the serum with staphylococci present in the scabs.

AUTHORS' SUMMARY.

PROTEINS OF RAGWEED POLLENS. C. A. JOHNSON and B. Z. RAPPAPORT, J. Infect. Dis. **50**:290, 1932.

Patients with autumnal hay fever do not react alike to the water-soluble protein fractions of giant and short ragweed pollens obtained by precipitation at various concentrations of ammonium sulphate. Rabbits treated with these preparations produced precipitins that reacted not only with the two ragweeds mentioned, but

also with extracts of rough marsh elder. Southern ragweed, cocklebur, slender ragweed, Western ragweed and bur ragweed.

Thirty-six patients subject to autumnal hay fever were tested by the cutaneous method with extracts of all of these pollens diluted to the same concentration of nitrogen. All reacted to short ragweed, thirty-five to giant ragweed, thirty-four to Western ragweed, thirty-three to slender ragweed, thirty-three to cocklebur, thirty to Southern ragweed and twenty-four to rough marsh elder. The short and giant ragweeds produced the largest, and the rough marsh elder, the smallest wheals. Immunologically, it would appear that these pollens are closely related; clinically, without attempting to explain differences in sensitivity in individual patients, the relationship is also apparent. While the data obtained from experiments on animals may be significant, we do not wish to imply that they are necessarily applicable to the symptom complex of hay fever. From evidence presented it would seem that the active principle of ragweed pollen is of protein nature, or that it clings to a protein moiety. A nitrogen-containing lipid was obtained from ragweed pollen by ether extraction, which gave distinctly positive reactions by the intradermal method in nineteen of twenty-one patients with autumnal hay fever. Other minor fractions of ragweed pollen are relatively inert.

C. A. JOHNSON.

THE STANDARDIZATION OF ANTIMENINGOCOCCIC SERUM BY THE POLYSACCHARIDE PRECIPITIN TEST. J. ZOZAYA, *J. Infect. Dis.* **50**:310, 1932.

This method of standardizing antimeningococcic serum gives direct evidence of the probable protective value of a given serum. Further clinical evidence is necessary to establish this assumption definitely. The agglutination test should, for the present, be continued with the object of determining the polyvalency of the serum, but not with that of judging its potency. Further work with the complex meningococcic polysaccharide may result in finding a fraction which is type-specific, and which can be substituted for the group-specific carbohydrate. Attempts are now being made to carry on this fractionation, as well as to determine the chemical composition of the complex group carbohydrate. This simple method should be given a thorough trial. Comparisons on a therapeutic basis should be made with serums containing different values of polysaccharide precipitable substance or unit value per cubic centimeter. Unfortunately, there is not a method at hand to test potency on animals, although the method suggested by Schwartzman may be of use in preliminary tests for comparison. The therapeutic dosage in terms of units would have to be established by experience, as is being done with the antipneumococcic serum. An advantage of this method is the constant control of the standard serum that can be supplied from Washington, for one can dilute or concentrate a given serum to give exactly the titration desired.

AUTHOR'S SUMMARY.

THE LEUKOCYTE RESPONSE IN MAN TO DICK TOXIN, WITH SPECIAL REFERENCE TO EOSINOPHIL CHANGES. S. L. VAUGHAN, *J. Infect. Dis.* **50**:315, 1932.

The eosinophil reaction had no apparent relation to the intensity of the Dick cutaneous reaction, to the rash or to the history of an attack of scarlet fever.

AUTHOR'S SUMMARY.

THE ANTIGENIC PROPERTIES OF RABIES VIRUS. L. C. HAVENS and C. R. MAYFIELD, *J. Infect. Dis.* **50**:367, 1932.

Specific flocculation of rabies virus occurs in appropriate dilutions of immune rabbit and guinea-pig serum. Flocculation occurs with fixed virus (rabbit brain) and with street virus. The serum of the rabies-immune guinea-pigs has been shown to possess specific complement-fixing antibodies for rabies virus. Immune rabbit serum is unsatisfactory for complement-fixation experiments with viruses, because of its anticomplementary nature.

AUTHORS' SUMMARY.

SOLUBLE SPECIFIC SUBSTANCES FROM YEASTLIKE FUNGI. H. D. KESTEN and E. MOTT, *J. Infect. Dis.* **50**:459, 1932.

A soluble polysaccharide fraction has been prepared from each of ten yeast-like fungi. Antiserums prepared against these fungi cause precipitation in high dilutions of the homologous soluble substances. In addition, cross-precipitation reactions are common. By absorption of precipitin on the mycotic bodies, however, the soluble substances exhibit definite specificity. Those obtained from *Saccharomyces*, *Willia* and *Monilia parapsilosis* are distinct among themselves and also from the remainder of those studied. The soluble substances from *Monilia albicans*, *Monilia psilosis* and *Endomyces albicans*, however, are serologically similar. This similarity is additional evidence for considering these as merely strains of a single species, *M. albicans*.

AUTHORS' SUMMARY.

THE RETICULO-ENDOTHELIAL APPARATUS IN INFECTIOUS DISEASES. K. M. DWOLAIZKAYA-BARYSCHEWA and N. W. KAGAN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:429, 1932.

The reticulo-endothelial system was eliminated by splenectomy and by blockade. The portal of entrance of *B. typhosus* and *B. paratyphosus* B had a distinct influence on the activity of the reticulo-endothelial system: in infections per os its defensive action is very insignificant, but becomes pronounced in subcutaneous infections.

I. DAVIDSOHN.

THE DEPENDENCE OF THE THERAPEUTIC EFFECT OF IMMUNE SERUMS ON THE INTACT RETICULO-ENDOTHELIAL SYSTEM. I. L. KRITSCHESKI, P. L. RUBINSTEIN and E. S. HERONIMUS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:463, 1932.

The full therapeutic effect of tetanus antitoxin and antipneumococcus serum was obtained only in healthy mice, while animals in which the reticulo-endothelial system was damaged by splenectomy and injections of certain colloids were not protected against injections of tetanus toxin and of pneumococci, type I.

I. DAVIDSOHN.

THE SPIROCHAETA PALLIDA REACTION AND ITS RELATION TO THE WASSERMANN REACTION. W. GAEHTGENS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **73**:527, 1932.

Because of its high sensitivity and specificity, an aqueous phenolized suspension of cultures of *Spirochaeta pallida* is recommended for the complement-fixation test for syphilis. Technical details of the test are given. Positive syphilitic serums contain at least two different types of reacting substances, as shown by proper absorption experiments: (a) those reacting with the *Spirochaeta pallida* antigen, and (b) those reacting with syphilitic liver. The Wassermann reaction and the *Spirochaeta pallida* reaction rest on two basically different serologic phenomena.

I. DAVIDSOHN.

## Tumors

THE ASSOCIATION OF TUBERCULOSIS AND CARCINOMA. F. G. COOPER, *Am. Rev. Tuberc.* **25**:108, 1932.

Several hundred reports of cases in which carcinoma and tuberculosis were present in the same organ are reviewed. In addition, twenty-four new cases are presented. All these revealed an intimate association of malignant growth and tuberculous lesion. Cooper concludes that the two diseases are not antagonistic. Tuberculosis may exist with tumors of all degrees of malignancy. Tuberculosis in an organ or biopsy specimen does not exclude the possibility of malignant tumor.

H. J. CORPER.



THE CLINICAL SIGNIFICANCE AND APPLICATION OF HISTOLOGIC GRADING OF CANCERS. W. C. HUEPER, *Ann. Surg.* **95**:321, 1932.

Sections removed for grading of malignancy must be taken from the peripheral zone of the tumor, must be properly prepared and stained, and must be evaluated by a pathologist familiar with the method of grading. Grading is helpful in the selection of the type of treatment. The grade must never interfere with the extent of the intensity of treatment. The grade indicates the proliferative and metastatic tendencies. A reliable prognostication must include consideration of at least three factors: the grade of malignancy, the extent and the location of the tumor.

AUTHOR'S SUMMARY.

THE BASOPHIL ADENOMAS OF THE PITUITARY BODY AND THEIR CLINICAL MANIFESTATIONS (PITUITARY BASOPHILISM). H. CUSHING, *Bull. Johns Hopkins Hosp.* **50**:137, 1932.

Of all subjects that engage the attention of the profession at the present day, that of endocrinology particularly lends itself to the temptation of impressionistic speculation. During the past ten years, innumerable syndromes of so-called polyglandular type, some of them bearing a certain resemblance to that under consideration, have often been described in print. Examples of "diabetes in bearded women," of rapidly acquired obesity, of hypertension, of masculinization in the female and of sexual precocity in children of either sex, often associated with hyperplasias or tumors of one sort or another of the suprarenal glands, have been so many and varied as to baffle analysis. Some of these syndromes have unquestionably been due to corticosuprarenal tumors, and in not a few instances, indeed, such a tumor has been removed at operation with definite amelioration of symptoms. What is more, in similar states suprarenal tumors have been found after death in the absence of any recognizable abnormality in the pituitary body, though all too often the protocol refers to the examination of this structure either in the briefest terms or not at all. While there is every reason to concede, therefore, that a disorder of somewhat similar aspect may occur in association with pineal, with gonadal or with suprarenal tumors, the fact that the peculiar polyglandular syndrome, which pains have been taken herein conservatively to describe, may accompany a basophil adenoma in the absence of any apparent alteration in the suprarenal cortex other than a possible secondary hyperplasia will give pathologists reason in the future more carefully to scrutinize the anterior lobe of the pituitary gland for lesions of similar composition.

AUTHOR'S SUMMARY.

INACTIVATION OF AGENT OF A CHICKEN TUMOR BY MONOCHROMATIC ULTRAVIOLET LIGHT. E. STURM, F. L. GATES and J. B. MURPHY, *J. Exper. Med.* **55**:441, 1932.

Even though part of the energy of the incident light is probably absorbed by chemical entities that play no part in the specific reaction of inactivation, nevertheless the wavelengths most active in destroying biologic cells or agents will presumably be among those absorbed in the highest proportion. This would indicate that the curves here presented are approximately reciprocal to the coefficients of absorption of particular substances, the destruction of which caused the inactivation of the agents or the death of the cells. The similarity between the curves for bacteria, virus and phage, both in shape and in total involved energies, suggests the presence of a common factor, or of closely related chemical entities, sensitive to ultraviolet rays, whereas the data for the tumor agent suggest that its inactivation is due to the destruction of a substance having an essentially different spectral absorption, and therefore of a different chemical character. While the amount of ultraviolet energy required to affect the tumor agent is great, it is

still less than that involved in the inactivation of some of the enzymes. A study is under way to compare the deduced spectral analysis with the actual coefficients of absorption of the highly purified tumor agent.

AUTHORS' SUMMARY.

EFFECT OF TESTICLE EXTRACT ON TRANSPLANTABLE MOUSE TUMORS. R. C. TANZER, *J. Exper. Med.* **55**:455, 1932.

Grafts of a transplantable mouse sarcoma designated as no. 180, and those of an attenuated strain of a more malignant sarcoma, S/37, treated with testicle extract, either fail to grow on inoculation or result in tumors of a lower rate of growth than that of the controls. Autografts of spontaneous mouse tumors so treated show little if any effect, while the Bashford adenocarcinoma and the unattenuated S/37 are unaffected. The factor in testicle extract responsible for the retarding activity passes readily through a Berkefeld filter and is thermostable.

AUTHOR'S SUMMARY.

PRIMARY ADENOCARCINOMA IN A MECKEL'S DIVERTICULUM. P. MICHAEL and H. G. BELL, *Surg., Gynec. & Obst.* **54**:95, 1932.

A primary adenocarcinoma in a Meckel's diverticulum, apparently the first to be reported, is described in a man 67 years of age. Seven instances of sarcoma of Meckel's diverticulum have been reported.

AUTOLYSIS IN MALIGNANT AND NORMAL RABBIT TISSUES. H. I. PRICE, *Biochem. J.* **25**:1491, 1931.

Robin, in an analysis of the composition of a series of tissues obtained at autopsy from persons with and without malignant tumors (*Bull. Acad. de méd., Paris* **81**:799, 1919), came to the conclusion that the proportion of hydrolyzed to unhydrolyzed protein was greater in the tissue adjacent to a malignant growth than in the growth itself or in the corresponding tissue of a normal person. Price repeated Robin's experiment, using rabbits inoculated with the Brown-Pearce tumor and having metastases in the liver and kidneys. Determinations of hydrolyzed and unhydrolyzed protein content, and of water content, were made on the tumor tissue, the adjacent normal tissue and distant normal tissue from the same organ, at intervals up to twenty-four hours from the moment of death. The highest proportion of hydrolyzed to unhydrolyzed protein, at the instant of death, was present in the tumor tissue, the lowest in the tissue most distant from the malignant growth. As the result of a gradual postmortem transfer of water, presumably containing dissolved, hydrolyzed protein, from the tumor area to the surrounding normal tissue, the proportion of hydrolyzed to unhydrolyzed protein in the tissue adjacent to the tumor gradually approached and finally exceeded that of the tumor itself (the balance observed by Robin). The tissue adjacent to the tumors contained a definitely higher proportion of hydrolyzed to unhydrolyzed protein than the tissue more distant from the tumors, during the entire interval of observation. No evidence was found, however, to support Robin's earlier conclusions that the tissues adjacent to a malignant growth may autolyze more rapidly than the corresponding tissues of a noncancerous person, and that the protein requirements of a tumor are met by the hydrolysis of the proteins of the surrounding tissue. The increased proportion of hydrolyzed protein in the tissues adjacent to a tumor may be absorbed from the disintegrating tumor tissue rather than vice versa.

ARTHUR LOCKE.

THE PHOSPHATIDE AND CHOLESTEROL CONTENTS OF NORMAL AND MALIGNANT HUMAN TISSUES. M. JOWETT, *Biochem. J.* **25**:1991, 1931.

Tumor tissues have a higher phosphatide and cholesterol content, and a higher phosphatide-cholesterol ratio, than the tissues in which they have their origin.

ARTHUR LOCKE.

DISEASE IN MICE TREATED WITH CARCINOGENIC AGENTS. J. M. and C. C. TWORT, *J. Path. & Bact.* **35**:219, 1932.

This article, which will be of special interest to workers using mice in cancer research, gives a review of the results of some 12,000 postmortem examinations of mice treated with carcinogenic and other agents.

PARATHYROID TUMORS WITHOUT OSTEITIS FIBROSA. C. HADFIELD and H. ROGERS, *J. Path. & Bact.* **35**:259, 1932.

Two instances of large parathyroid adenoma are described, in one of which there was a normal skeleton, while in the other there was acromegaly associated with a large chromophil adenoma of the pituitary gland. These cases, as well as three others from the literature, illustrate the fact that a parathyroid tumor, closely resembling the normal gland in structure, may occur without elaborating any excess of internal secretion.

CARCINOMA OF THE PITUITARY GLAND WITH ABDOMINAL METASTASES. M. D. GILMOUR, *J. Path. & Bact.* **35**:265, 1932.

Gilmour reports a case of adenocarcinoma of the pituitary gland with multiple metastases. Lesions in the thyroid gland and ovary are described, and it is considered that these lesions and the pituitary disease are interrelated.

IMMUNITY TO JENSEN'S RAT SARCOMA PRODUCED BY TUMOUR EXTRACTS. H. CHAMBERS and G. M. SCOTT, *J. Path. & Bact.* **35**:283, 1932.

Experiments are described which show that Jensen's rat sarcoma, deprived of its blood supply and kept at blood temperature, undergoes a transitory change during which extracts from it have immunizing properties. The immunizing property develops for a short time with increasing potency and then disappears; it is apparently due to changes in the tumor cells set up by defective oxygenation.

AUTHORS' SUMMARY.

THE CLASSIFICATION OF CANCER OF THE RECTUM. C. E. DUKES, *J. Path. & Bact.* **35**:323, 1932.

Cancers of the rectum can be divided into *A*, *B* and *C* cases according to the extent of spread. *A* cases are those in which the growth is limited to the wall of the rectum; *B* cases, those in which there is extrarectal spread but no lymphatic metastases; *C* cases, those in which metastases are present in the regional lymph nodes. A striking difference is found in the operative mortality and in the period of survival after operation in these three groups. There is reason to believe that in *A* cases the disease is completely eradicated by rectal excision, and the excellent results of operative treatment confirm the opinion previously expressed that lymphatic metastases are not found until a rectal carcinoma has spread by direct continuity to the extrarectal tissues. A good prognosis is justified also in *B* cases, though slightly less favorable than in *A*. The results of surgical treatment in *C* cases are disappointing. The scope and limitations of histologic grading by Broders' method are discussed, and the conclusion is reached that grading of a tumor is also of value for prognosis, though not when applied to fragments removed for diagnosis.

AUTHOR'S SUMMARY.

A MALIGNANT MELANOTIC TUMOUR OF GANGLION CELLS ARISING FROM A THORACIC SYMPATHETIC GANGLION. W. G. MILLAR, *J. Path. & Bact.* **35**:351, 1932.

A case is described of a very malignant tumor composed of cells of nervous origin the structure of which approximated, in a greater or lesser degree, that of ganglion cells. The more highly differentiated ganglion cells were loaded with

pigment; this was melanin and was present in sufficient quantity to make parts of the tumor as black as an ordinary cutaneous melanoma. Evidence is adduced that the tumor arose from nerve cells in the seventh left thoracic sympathetic ganglion. In view of the undoubted nervous origin of this tumor it is both possible and likely that a similar origin can be postulated for some at least of the melanomas arising in the suprarenal medulla.

AUTHOR'S SUMMARY.

### Medicolegal Pathology

LETHAL ACCIDENT DUE TO INHALATION OF TRICHLORETHYLENE. R. PFREIMBTER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:339, 1931.

The now frequent use of trichlorethylene ( $\text{CHCl}:\text{CCl}_2$ ), instead of benzene, for cleaning metal or for extraction of fat substances has led to fatal poisonings; twenty-four such instances have been reported. A case involving an 18 year old mechanic is described. Autopsy showed vesicle-like formations in the skin of the lower parts of the arms and legs with a marginal zone of hyperemia, presenting a burnlike effect. On slicing the brain, a sweetish odor, similar to that of chloroform, was noticed. Death occurred from asphyxiation due to aspiration of the gastric contents into the respiratory system while vomiting during unconsciousness. The liver showed toxic changes of the hepatic cells and deposits of pigments due to blood lysis. Eosinophils were increased in number in the peripheral blood. This case illustrates the danger of inhalation of trichlorethylene, even in a cool room where the oxygen content of the air is sufficient. In prolonged exposures, it might cause partial blindness from degenerative changes of the optic nerve and also damages to the sensory branches of the trigeminus. The chronic use of this drug may lead to acute yellow atrophy of the liver.

E. L. MILOSLAVICH.

AN ERRONEOUSLY INTERPRETED GUNSHOT WOUND OF THE SKULL. K. WALCHER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:345, 1931.

While riding on a motorcycle, a man was shot in the back of the head, with the outlet in the frontal region and extensive fracturing of the skull. Both wounds, entrance and exit, were irregular and led the examiners to assume erroneously that the inlet was in the right temporal region. The assailant pleaded self-defense, which was refuted later on by the subsequent correct anatomic interpretation of the wounds as having been produced by discharge of a gun from behind, at close range.

E. L. MILOSLAVICH.

CHEMICAL ANALYSIS OF GUNSHOT WOUNDS. O. SCHMIDT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:353, 1931.

In examining a gunshot injury, one should first remove the visible unburnt particles of powder and subject them to the diphenylamine sulphuric acid test. A positive reaction alone might prove that a pistol was fired at close range. However, should the results be doubtful or should there be question as to the character and type of the bullet or uncertainty as to whether the perforation is the inlet or the outlet wound, chemical examinations are instituted. The ring of contusion must be examined separately. Presence of minute traces of metallic particles farther away from the bullet wound means a shot fired at close range; consequently it indicates the entrance of the bullet. If the inlet and outlet wounds disclose presence of lead, a lead bullet was used. Absence of any lead proves penetration by a jacketed projectile. If the latter, however, was smashed into small pieces, such fragments may leave traces of lead in the exit wound. Evidence of lead and mercury in the ring of contusion in wounds produced by discharge of a gun at a great distance indicates use of Flobert ammunition, and the site of deposits of mercury points to the entrance of the discharge. In chemical examina-

tions of gunshot perforations of clothing for antimony, one should keep in mind that antimony is used in the textile industry for preparation of fabrics, and therefore control tests should not be omitted.

E. L. MILOSLAVICH.

SPERM CRYSTALS. E. ZIEMKE, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:367, 1931.

The most commonly used tests in examinations for sperm are the Florence iodine reaction and the Barberio trinitrophenol test. Niederland (*Med. Welt* **5**:149, 1931) recently described a new microchemical reaction: If sulphuric acid is added to a drop of sperm, numerous crystals in the form of prismatic needles or rods soon develop and can be observed by the naked eye or by help of a magnifying glass. The crystals are insoluble in cold or warm water, ether, alcohol or chloroform. In testing for sperm on clothing, the spot is macerated in water for from three to four hours. To a drop of the extract on a slide one drop of a 3 per cent solution of sulphuric acid is added. The crystals develop after a few minutes; in very thin extracts, after a few hours. The formed crystals remain unchanged for several months. The crystals can be obtained even from disintegrated sperm, when the Florence and Barberio reactions usually fail to show any results. Other bodily fluids and secretions, such as nasal mucus, sputum, gonorrheal pus and vaginal secretion, yield a positive sulphuric acid reaction, but the results of the Florence and Barberio tests in these cases are always negative. Experiments made with these three tests disclosed that the Niederland reaction is the most reliable one, while the Florence test gave results in 70 per cent of the cases examined and the Barberio test in only 26.3 per cent. The ready development of the crystals, which consist of calcium phosphate, by application of sulphuric acid is explained by the abundant calcium content of sperm. If one adds a 3 per cent solution of sulphuric acid to a solution of calcium chlorate, the same kind of crystals results. Sperm contains in 900 parts of water, 100 parts of solid material, of which 60 parts are organic and 40 inorganic substances. In the last mentioned, 30 parts are represented by calcium phosphate. The practical value of this test is limited, since all the fluids and secretions containing calcium give the same result. But an absence of the sulphuric acid reaction positively eliminates presence of sperm. Should the reaction yield a positive result, a subsequent examination of the rest of the same material with the Florence test should follow. The Niederland test is an important addition to the already existing sperm reactions and serves as a general, reliable orientation, but cannot replace the Florence test.

E. L. MILOSLAVICH.

TANGENTIAL GUNSHOT WOUNDS OF THE SKULL. R. M. MAYER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:419, 1932.

Atypical gunshot wounds result if a projectile strikes the convexity of the skull in a slanting direction. Thus the external and internal tables become irregularly fractured and detached, and the tiny fragments of bone appear raised. From the perforation made by the bullet, fracture lines extend in radial or star-shaped fashion. The angle at which the bullet penetrated can be reconstructed from the slope of the edge of the wound. However, one has to consider the position of the body or of the head at the time of the discharge of the pistol, and also the possibility that the bullet might have been deflected from its course, striking the skull transversally.

E. L. MILOSLAVICH.

SPONTANEOUS COMBUSTION OF THE HUMAN BODY. ERNST DARMSTAEDTER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:437, 1932.

This is a critical review of the literature of the last century, with a discussion of the physicochemical problems involved. The article, which is of historical interest, should be read in the original.

E. L. MILOSLAVICH.

## Book Reviews

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**Antony van Leeuwenhoek and His "Little Animals."** Being Some Account of the Father of Protozoology and Bacteriology and His Multifarious Discoveries in These Disciplines. Collected, Translated and Edited From His Printed Works, Unpublished Manuscripts and Contemporary Records by Clifford Dobell, F.R.S., Protistologist to the Medical Research Council, London; Foreign Member of the R. Accademia dei Lincei, Rome; Sometime Fellow of Trinity College, Cambridge. Published on the Three Hundredth Anniversary of His Birth [Oct. 24, 1632]. Pp. 435, with 32 plates. Price, \$7.50. New York: Harcourt, Brace and Company, 1932.

This remarkable book begins with an epistle to the reader in which the author explains how the book came to be written. "It is now some 25 years since I first began to try and find out something about Leeuwenhoek and his discoveries in protozoology and bacteriology. The task has always been hard, but because of my personal interest it has never been irksome. My interest has, indeed, grown with my knowledge, and the more I have found out, the more I have ever wanted to find out about this truly marvelous man and his works. From the very beginning, I have been able to get little or no help from the writings of others (most of whom merely led me astray), so that I have always had to do the best I could for myself." After many setbacks, Dobell found that all of Leeuwenhoek's original writings were in the form of letters in colloquial Dutch, and that these letters for the most part are extant in the archives of the Royal Society in London. Now followed years of effort to learn to read Leeuwenhoek's script before the contents of the letters could be mastered. In studying the numerous letters much was learned about the man himself, his character and his work in general. Apparently search has been made also of every other accessible source of information, and the result is an account of surpassing interest of the life of Leeuwenhoek and his work in protozoology and bacteriology. It is obvious, as the author himself points out, that the making of this book has been a labor of love.

The account of Leeuwenhoek's life occupies pages 19 to 105. Aside from his apprenticeship of six years to a draper in Amsterdam his days were spent in Delft, where he died in 1723, at more than 90 years of age. In 1654 he set himself up as draper and haberdasher. Plate V is a facsimile bill in Leeuwenhoek's writing. He had no formal, higher education. He had not, as is sometimes stated, any sort of medical or scientific training. He knew only one language, the Dutch of his time, in which he expressed himself in simple fashion. In 1673, apparently without any warning, he offered a letter for publication in the transactions of the Royal Society of London. This letter dealt with microscopic observations on mold, on the bee and on the louse. Dobell tells us that this letter was sent at the instance of Régnér de Graaf, of graafian follicle fame, a friend and fellow-townsmen of Leeuwenhoek, and one of the many correspondents of the energetic Henry Oldenburg, the first secretary of the Royal Society. From this time on until his death, fifty years later, Leeuwenhoek sent letters to the Royal Society, which "cover an immense field, and contain observations on matters zoological, botanical, physical, physiological, and miscellaneous (unclassifiable). They are mostly—but not entirely—concerned with observations and discoveries made with the microscope." Even on his death bed he sent two letters. In 1680 Leeuwenhoek was made a member of the Royal Society. His observations were made by means of microscopic lenses of his own making. He left behind him about two hundred and forty-seven microscopes. He worked entirely by himself. How he succeeded in seeing what he described in his letters is a mystery, toward the solution of which Dobell offers pertinent suggestions. All these matters and much more—his visitors, the impression that he made on his contemporaries, the devotion of his daughter,

Maria, the various translations and publications of his letters (he wrote no books or formal papers), his health, etc.—are set forth with abundant documentation in the light of the history of his own time. On one weighty point the chronicle is silent, namely: Just how did it happen that Leeuwenhoek began to grind lenses and make observations with his microscopes? All that can be said in answer is that he worked in response to a craving after knowledge. How the start was made and why is not known.

Pages 102 to 299 contain, with comments and annotations, the translations into English by Dobell of Leeuwenhoek's original letters in Dutch, in which are recorded his observations on "little animals"—protozoa and bacteria, free-living as well as entozoic. Facsimiles are given of parts of these letters. In the translation an effort was made to preserve the flavor of Leeuwenhoek's writing and to meet also the requirements of modern protistology. One example, chosen at random, may be given: In letter 110, in the midst of a discussion of the eggs of snails, the germination of wheat and the spat of oysters, Leeuwenhoek wrote that he mixed stuff from the hollows in the roots of one of his teeth, which he had removed because it was loose, "with clean rain-water and set it before my magnifying-glass so as to see if there were as many living creatures in it as I had aforetime discovered in such material: and I must confess that the whole stuff seemed to me to be alive. But notwithstanding the number of these animalcules was so extraordinarily great (though they were so little withal, that 'twould take a thousand million of some of 'em to make up the bulk of a coarse sand-grain, and several thousands were a-swimming in a quantity of water that was no bigger than a coarse sand-grain is), yet their number appeared even greater than it really was: because the animalcules, with their strong swimming through the water, put many little particles which had no life in them into like motion, so that many people might well have taken these particles for living creatures too." It will be sufficient to say further that after years of patient labor Leeuwenhoek's descriptions of protozoa and bacteria now have been rendered into English by a scientist and scholar of unique competence for the task. It is astonishing how many microbic forms Leeuwenhoek described so clearly that they can be recognized today. It is noteworthy also that he confined himself to plain, objective descriptions without speculation, and that he did not associate his "little animals" with the causation of disease.

The next sixty pages deal with elucidations and annotations concerning various topics: Leeuwenhoek's name, his language, his microscopes and microscopic methods, his dwelling, his draughtsmen, his portraits, his seals and his "first 27 unpublished letters." These letters were regarded as lost, but most of them have been found by Dobell among the manuscripts in the Royal Society.

In his envoy Dobell discusses comprehensively and critically Leeuwenhoek's place in protozoology and bacteriology. Before Leeuwenhoek there was speculation and prophecy only in regard to microbes; before him nobody saw a protozoon or a bacterium with his own eyes; consequently he alone is entitled to the distinction of being called the father of protozoology and bacteriology. "Leeuwenhoek will be finally judged by his own writings, and not by anything that other people say he wrote. He has left us a great mass of records—both published and unpublished—from which we can now extract what we please. I have endeavored to recover from them all his observations on the Protozoa and the Bacteria, and to set in order his inchoate and uncorrelated findings in a manner which may fairly convey their import and importance to present-day students. To me his words, when judiciously weighed in the scales of contemporary and recent knowledge, prove conclusively that he was the first protozoologist and the first bacteriologist."

The volume, an admirable and attractive example of appropriate bookmaking, concludes with a short list of Leeuwenhoek's writings—manuscripts and publications—a list of other references and sources and an index.

Dobell has made an important addition to the history of science. We have only a few books about scientific men and their work as interesting as his book about Leeuwenhoek.

**Classic Descriptions of Disease.** By Ralph H. Major, M.D., Professor of Medicine, University of Kansas School of Medicine. Price, \$4.50. Pp. 630, with 130 illustrations. Springfield: Charles C. Thomas, 1932.

This interesting book is a sister volume to Long's "Readings in Pathology" and to Fulton's "Selected Readings in the History of Physiology." It was a happy idea that led the editor to gather the selections and arrange them for publication, which has been carried out commendably by the publisher. The book contains selected original descriptions of disease from Hippocrates down to the present day, with brief historical summaries and biographic sketches by the editor. It is essentially an anthology of nosography. There are reproductions of portraits, many unusual, of title and text pages of old books, and of drawings and apparatus. In all there are 376 selections from 179 writers of outstanding contributions to the knowledge of disease. Of the nonmedical writings included may be mentioned accounts by Boccaccio, Kircher and Defoe of the plague. American medicine is well represented. No fault may be found with the selections, and the field has by no means been exhausted. Treatment has not been included; neurology, ophthalmology, dermatology and other special fields are omitted. In the main, the selections have been culled from writings in the general field of so-called clinical medicine. Vaccination is not represented, and if ever a second edition appears it by all means should include Edward Jenner's classic account of allergy in smallpox and vaccinia, a pioneer description of allergy in infectious diseases. The recent descriptions by physicians of undulant fever, Rocky Mountain spotted fever and tularemia also should receive consideration. The book is divided into ten sections: infectious diseases, diseases of metabolism, lead poisoning, diseases of the circulatory system, diseases of the blood, renal diseases, respiratory diseases, deficiency diseases, allergic diseases and diseases of the digestive tract. Except when otherwise indicated, the editor is responsible for the translations into English. This book will have a special appeal to physicians who are interested in the historical development of the knowledge of disease, and it should be made easily available for students of medicine because it provides direct and stimulating contact with the founders of nosography.

**The Wisdom of the Body.** By Walter B. Cannon, M.D., Sc.D., LL.D., George Higginson, Professor of Physiology, Harvard Medical School. Price, \$3.50. Pp. 312. New York: W. W. Norton & Company, Inc., 1932.

With this volume, the author enters the quasi-popular field. The essence of the subject matter is contained in a word of the author's coinage, "homeostasis," a term by which he designates the processes that preserve physiologic stability in the living organism. From the large number of such forms of "homeostasis," those selected for detailed discussion are the ones with which the author has been most intimately concerned, namely, those related to the functions of the "sympathico-adrenal" mechanism. Thus, hunger, thirst, hemorrhage and shock, the fluctuations in blood sugar and certain aspects of the physiology of respiration receive emphasis, while other processes, for example the responses of the body to the challenges of invading micro-organisms, are much more briefly dealt with.

Professor Cannon's book enjoys the distinction of few works of the kind in being doubly authoritative. The distinguished position of the author in the scientific world might have permitted him to present many concepts *ex cathedra* without detracting from the value of the work so far as the average lay reader is concerned. The author has not relied on such a method. For the most part, he has followed the manner of the technical journals, citing previous experimenters by name and describing in detail many of his own researches, before stating conclusions. Whether or not he has thus fully succeeded in presenting the material in a manner intelligible to "anyone who has had a simple training in biology and in general science" must remain for each reader to determine. Since writers on popular science have so frequently been lured away from strict facts by the



temptation to dramatize their subject, discerning readers have in this book an opportunity for unusual satisfaction and refreshment. The numerous charts are helpful. Each chapter is followed by a short bibliography, and there is appended a list of the publications of the author and his co-workers and pupils on which the book is based.

**Vitamins: A Survey of Present Knowledge.** Compiled by a Committee appointed jointly by the Lister Institute and Medical Research Council. Medical Research Council, Special Report Series No. 167. Price, 6 shillings, 6 pence, net. Pp. 319. London: His Majesty's Stationery Office, 1932. (Can be obtained from The British Library of Information, 5, East Forty-Fifth Street, New York.)

This book is the third edition of a report about the knowledge concerning vitamins by a committee of the Medical Research Council published in 1919. The survey now presented "is an entirely fresh resumption of current knowledge and of technical methods in this subject." The committee in charge of the survey is composed of leading English workers in the vitamin field, under the chairmanship of E. Mellanby. The general editor is Arthur Harden. An enumeration of the chapter headings will indicate the scope of the report: historical introduction; the fat-soluble vitamins, vitamin A; vitamin D (the antirachitic, calcifying vitamin); vitamins and dental tissues; vitamin E; the vitamin B complex; pellagra as a vitamin deficiency disease; vitamin C, the antiscorbutic vitamin; some nutritional aspects of cow's milk with special reference to vitamins; vitamins and human diets; vitamins in relation to the diet of the mother and the infant. The references, arranged alphabetically according to authors, cover about twenty-five pages. The titles to articles in periodicals are omitted, which will please those interested in the economics of scientific printing. Appendix 1 consists of a table giving the distribution of vitamins in foodstuffs. Appendix 2 contains a report of the conference on vitamin standards in London in June, 1931. At the end is a complete index. The survey as a whole is an admirable and complete summary of the development and present state of knowledge of the vitamins. The historical introduction and historical summaries elsewhere are models of their kind. The survey will be of great help to students in the vitamin field everywhere. Its manifest usefulness should tend to overthrow the statement in the preface that further revising may not be justifiable. It is hard to believe that the publication as proposed of a new journal, *Nutrition Abstracts and Reviews*, will meet fully the need for systematic and comprehensive summaries of the order of this survey.

**The Sputum: Its Examination and Clinical Significance.** By Randall Clifford, M.D., Associate in Medicine, Peter Bent Brigham Hospital; Assistant in Medicine, Harvard Medical School. Price, \$4. Pp. 167, with 21 figures and 7 plates in colors. New York: The Macmillan Company, 1932.

This book is offered as a practical guide in the examination of the sputum and in the clinical interpretation of the results. It aims to meet the needs of physicians and medical students in their own work. There are four sections. The first deals with the sputum in general and with practical points in its collection. The second section describes macroscopic examination: physical characteristics, gross constituents and chemical features. The third section deals with microscopic examination: the unstained smear, the Ziehl-Neelsen method of staining for tubercle bacilli, Smith's gram-eosin stain and the Fontana stain. In the fourth section the character and clinical significance of the sputum in common diseases of the bronchi and lungs is discussed. The illustrations, twenty-one in black and white and seven in colors, are appropriate and helpful. The book is an authoritative guide to the microscopic examination of the sputum for clinical purposes.

## Books Received

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HANDBUCH DER BLUTGRUPPENKUNDE. Bearbeitet von Dr. H. Bürkle-de la Camp, Privatdozent, Oberarzt der chirurgischen Universitätsklinik München; Dr. M. Hesch, Assistent am anthropologisch-ethnologischen Forschungsinstitut der Universität Leipzig; Dr. G. Raestrup, o. Professor, Direktor des Instituts für gerichtliche Medizin der Universität, Frankfurt a. M.; Dr. E. D. Schött, Facharzt für Haut- und Geschlechtskrankheiten; Dr. P. Steffan, Marinegeneraloberarzt, Chefarzt des Marine-lazarets, Wilhelmshaven; Dr. O. Thomsen, o. Professor, Direktor des Instituts für allgemeine Pathologie, Kopenhagen; I. S. Wellisch, Senatsrat, Wien. Herausgegeben von Dr. Paul Steffan, Marinegeneraloberarzt und Chefarzt des Marine-lazarets, Wilhelmshaven. Mit 125 Abbildungen und 3 Karten. Price, paper, 48 marks; bound, 50 marks. Munich: J. F. Lehmann, 1932.

ESSENTIALS OF PATHOLOGY. By C. Russell Salsbury, M.D., C.M., Professor of Anatomy, University of Oklahoma. Price, \$2. Pp. 270. New York: The Macmillan Company, 1932.

THE CARDIAC OUTPUT OF MAN IN HEALTH AND DISEASE. By Arthur Grollman, Ph.D., M.D., Associate Professor of Physiology in the Medical School of the Johns Hopkins University. Price, \$4. Pp. 324, with 25 figures. Springfield, Ill.: Charles C. Thomas, 1932.

INDIVIDUALITY OF THE BLOOD IN BIOLOGY AND IN CLINICAL AND FORENSIC MEDICINE. By Prof. Leone Lattes, Director of the Institute of Forensic Medicine in the University of Modena. Translated by L. W. Howard Bertie, M.A., B.M., B.Ch. (Oxon.). Revised from the French edition of 1929. Price, \$7.50. Pp. 413. New York: Oxford University Press, 1932.

CHEMISTRY OF THE OPIUM ALKALOIDS. By Lyndon F. Small, Consultant in Alkaloid Chemistry, United States Public Health Service, University of Virginia. Assisted by Robert E. Lutz, Associate Professor of Chemistry, University of Virginia. Prepared by Direction of the Surgeon General. Pp. 375. Supplement No. 103 to the Public Health Reports. Washington, D. C.: Superintendent of Documents, 1932.

THE HAEMOLYTIC STREPTOCOCCI: THEIR GROUPING BY AGGLUTINATION. By Frederick W. Andrewes and Ethel M. Christie. Medical Research Council, Special Report Series, No. 169. Price, 1 shilling 3 pence, net. Pp. 73. London: His Majesty's Stationery Office, 1932.

STUDIES IN THE PSYCHOLOGY OF DELINQUENCY. By Grace W. Pailthorpe. Medical Research Council, Special Report Series No. 170. Price, 2 shillings, net. Pp. 113. London: His Majesty's Stationery Office, 1932.

APPLIED BACTERIOLOGY. By Thurman B. Rice, A.M., M.D., Professor of Bacteriology and Pathology, Indiana University School of Medicine and Training School for Nurses. Price, \$2.50. Pp. 276. New York: The Macmillan Company, 1932.

MEDICINA FENNICA VII ANNO MCMXXXI. Edidit Societas Medicorum Fennica Duodecim. Pp. 226. Helsinki: 1932.

BEITRÄGE ZUR KLASSIFIZIERUNG DER TUMOREN DES MUNDES UND SEINER NEBENORGANE. Inaugural Dissertation. Herman Richard Churchill, Pp. 48. Rostock: Carl Hinstorffs Hofbuchdruckerei, 1932.

TUBERCULOUS DISEASE IN CHILDREN: ITS PATHOLOGY AND BACTERIOLOGY. By John W. S. Blacklock. Medical Research Council, Special Report Series No. 172. Price, 3 shillings, net. Pp. 155. London: His Majesty's Stationery Office, 1932.

MIKROBIOLOGISCHE UND IMMUNOLOGISCHE FORSCHUNGEN UNTER ANWENDUNG DER GEWEBEZÜCHTUNG. Von Prof. Dr. Ren Kimura Direktor des Mikrobiologischen Institutes der Kaiserlichen Universität zu Kyoto. Paper. Pp. 97, mit 2 Kurven im Text und 4 Tafeln. Kyoto, Isseido: 1932.

STUDIES FROM THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF PENNSYLVANIA. Edited by E. B. Krumbhaar. Volume 3. 1931-1932.

## APPENDICITIS IN MEASLES

I. DAVIDSOHN, M.D.

AND

JACOB M. MORA, M.D.

CHICAGO

Warthin<sup>1</sup> recently reported the finding of giant cells in the tonsils and pharyngeal mucosa of four patients who were in the prodromal stage of measles. He considered the change in the organs mentioned to be pathognomonic. A cervical lymph node removed from one of the patients showed no such change.

In his review of the literature up to the time of his publication, he referred to an article by Ewing<sup>2</sup> in 1909 and to one by Mallory and Medlar<sup>3</sup> in 1920 on the changes in the skin and mucous membranes in measles. None of these authors noted the occurrence of giant cells in the mucous membranes. Commenting on their findings, Warthin stated:

In measles either there is a difference in the pathology of the lesion in the mucous membranes of the tonsil and pharynx from that of the buccal mucosa or these investigators missed what is to my mind the most striking feature of the lesion in the tonsil and pharyngeal mucosa. It seems most probable that they failed to recognize it.

Warthin was apparently unaware of the fact that the findings in the tonsils in the course of measles to which he attributed such great importance were observed and reported twenty years before his publication by Alagna,<sup>4</sup> who, in 1908, studied eight children, from 1 to 4 years old, who had died of measles, and reported his findings in 1911. He limited his study to the changes in the nose and throat, the other organs being studied by Ciaccio,<sup>5</sup> whose findings will be mentioned later. Alagna observed in the tonsils a change not seen in the normal organs. Without any definite topographic relationship, masses of nuclei were seen, consisting of groups of from eight to fifteen lying together, each with a distinct nuclear membrane. They resembled megakaryocytes. The picture described by Alagna is undoubtedly of the same nature as that observed and described by Warthin.

From the Pathological Laboratories and the Department of Surgery, Mount Sinai Hospital.

1. Warthin, A. S.: Arch. Path. **11**:864, 1931.

2. Ewing, James: J. Infect. Dis. **6**:1, 1909.

3. Mallory, F. B., and Medlar, E. M.: J. M. Research **41**:327, 1920.

4. Alagna, G.: Arch. f. Laryng. u. Rhin. **25**:527, 1911.

5. Ciaccio, Carmelo: Virchows Arch. f. path. Anat. **199**:378, 1910.

In the histologic studies on cases of measles published after 1911 there is no reference to the finding of giant cells in the tonsils until Warthin's publication. The report of Ciaccio<sup>5</sup> in 1910 was based on a study of the same eight cases of measles the rhinolaryngologic aspects of which formed the basis of Alagna's report. In Ciaccio's report, no mention is made of an examination of the intestines and particularly of the appendix. There is a casual note on the rare occurrence of cells resembling megakaryocytes in the lumina of new-formed capillaries in the interstitial tissue of the lungs. Secher,<sup>6</sup> in 1916, reported the autopsy findings in two persons who had died of enteritis during the course of measles. In his review, he quoted the report of Alagna, but did not state whether he had examined the tonsils in his own cases, nor did he mention an examination of the appendix. In the intestinal tract, the lymphatic tissues were swollen, particularly Peyer's patches, which resembled those seen in typhoid fever, but they were not ulcerated. There is a detailed microscopic report of the changes in the lymphatic tissue of the intestinal tract, but giant cells were not noted. Abramow<sup>7</sup> observed giant cells in the skin of patients who had died during the course of measles. He did not mention details concerning the structure of the giant cells, but a picture shows that they do not in the least resemble the giant cells seen in the tonsils. In Abramow's cases, the giant cells were not seen before the fourth day after the appearance of the rash.

After Warthin's article was published, Finkeldey<sup>8</sup> reported, in 1931, the finding of the characteristic giant cells in the tonsils removed operatively from a child 7 years old in whom clinical measles appeared three days after the operation. He referred to the publication of Alagna in 1911, but emphasized the fact that in his case the changes in the tonsils were already present in the prodromal stage of the disease, while in the cases of Alagna the tonsils were examined after the patients had died as a result of the disease. The same difference holds true for the four cases of Warthin.

Herzberg<sup>9</sup> reported in January of this year the finding of similar giant cells in the lymph follicles of an appendix that was removed from a child 6 years old. Four days following operation, a typical measly rash developed. Herzberg's report seems to be the first in the literature with a mention of the finding of giant cells in the appendix in the prodromal stage of measles.

The observation of a similar case in the Mount Sinai Hospital prompted us to place it on record.

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6. Secher, K.: *Berl. klin. Wchnschr.* **53**:250, 1916.

7. Abramow, S.: *Virchows Arch. f. path. Anat.* **232**:1, 1921.

8. Finkeldey, W.: *Virchows Arch. f. path. Anat.* **281**:323, 1931.

9. Herzberg, Mortimer: *J. A. M. A.* **98**:139, 1932.

CASE 1.—A boy 6 years old was admitted to the Mount Sinai Hospital in the service of Dr. H. M. Richter on May 17, 1929. His temperature on admission was 100 F.; his pulse rate, 112; his respiration rate, 24. There was pain in the right lower quadrant of the abdomen, accompanied with nausea and vomiting. The mother stated that the condition started on the evening before admission, with generalized abdominal pain, which then localized in the right lower iliac region and was associated with vomiting. Two days before that there was marked swelling of the cervical glands on the left side, which subsided on the following day. On physical examination there were slight congestion of the pharyngeal mucosa and tenderness in the right iliac region. The white blood cell count was 17,800, with 77 per cent polymorphonuclear leukocytes. The diagnosis of acute appendicitis was made, and the patient was operated on within one hour after admission. Dr. Richter, who performed the operation, described the appendix as being "large, thick, bulbous at distal end, soft, fluctuating and filled with pus." The diagnosis of the pathologist was "gangrenous appendicitis."

On the day following operation, the temperature began to rise, reaching 105.2 F. in the evening; the pulse rate was 138 and the respiration rate 40. Congestion of the mucous membranes of the mouth and swelling of the left cervical glands were noted. A moderate cough developed, with râles in the bases of the lungs. On the morning of the third postoperative day a rash appeared. The diagnosis of measles was made. The further course of the measles and the postoperative recovery were uneventful, and the child left the hospital on the ninth day following admission.

Reexamination of the histologic sections, prompted by the report of Herzberg, revealed changes as follows: The lymph follicles showed the central cell exhaustion mentioned by the previous authors. Numerous giant cells were present having the same appearance as those seen in the photographs published by Warthin, Finkeldy and Herzberg. They varied in size, in the number of the nuclei and in the distinctness of the surrounding protoplasm.

Herzberg emphasized in his report that he did not see migration of the giant cells into the mucosa. In our case, giant cells were present in the mucosa in considerable numbers, particularly in the subepithelial layer (fig. 1 C). Some were seen passing through the epithelial lining. The lumen was filled with an exudate consisting of neutrophilic leukocytes and fibrin. In the midst of it there was a clump of nuclei strongly suggesting a giant cell similar to the smaller variety seen in the mucosa. The mucosa and all the other layers were densely infiltrated by neutrophilic leukocytes. The finding of acute suppurative appendicitis coincident with the changes characteristic of measles is of considerable practical interest, emphasizing that appendiceal symptoms during the course of measles may be due to actual inflammation of the organ, requiring surgical interference, and that they should not be discounted lightly as being a part of the general clinical picture of measles.

Since this case was presented at the meeting of the Chicago Pathological Society, we have had the opportunity to study three additional cases. In the second case, the appendix was removed two days after the appearance of the cutaneous eruption of measles and at least four days after the onset of photophobia. The third was a case of appendicitis occurring during the prodromal stage of measles. In the fourth case, the appendicitis appeared, and the operation was performed, following recovery from measles.

CASE 2.—A boy 8½ years old was admitted to the Contagious Disease Department of the Cook County Hospital, in the service of Dr. B. M. Gasul, on April 27, 1932. He had been well till three days before admission; then a cough developed, followed by photophobia, and for the twenty-four hours preceding his admission to the hospital he experienced severe generalized abdominal pain, which became local-

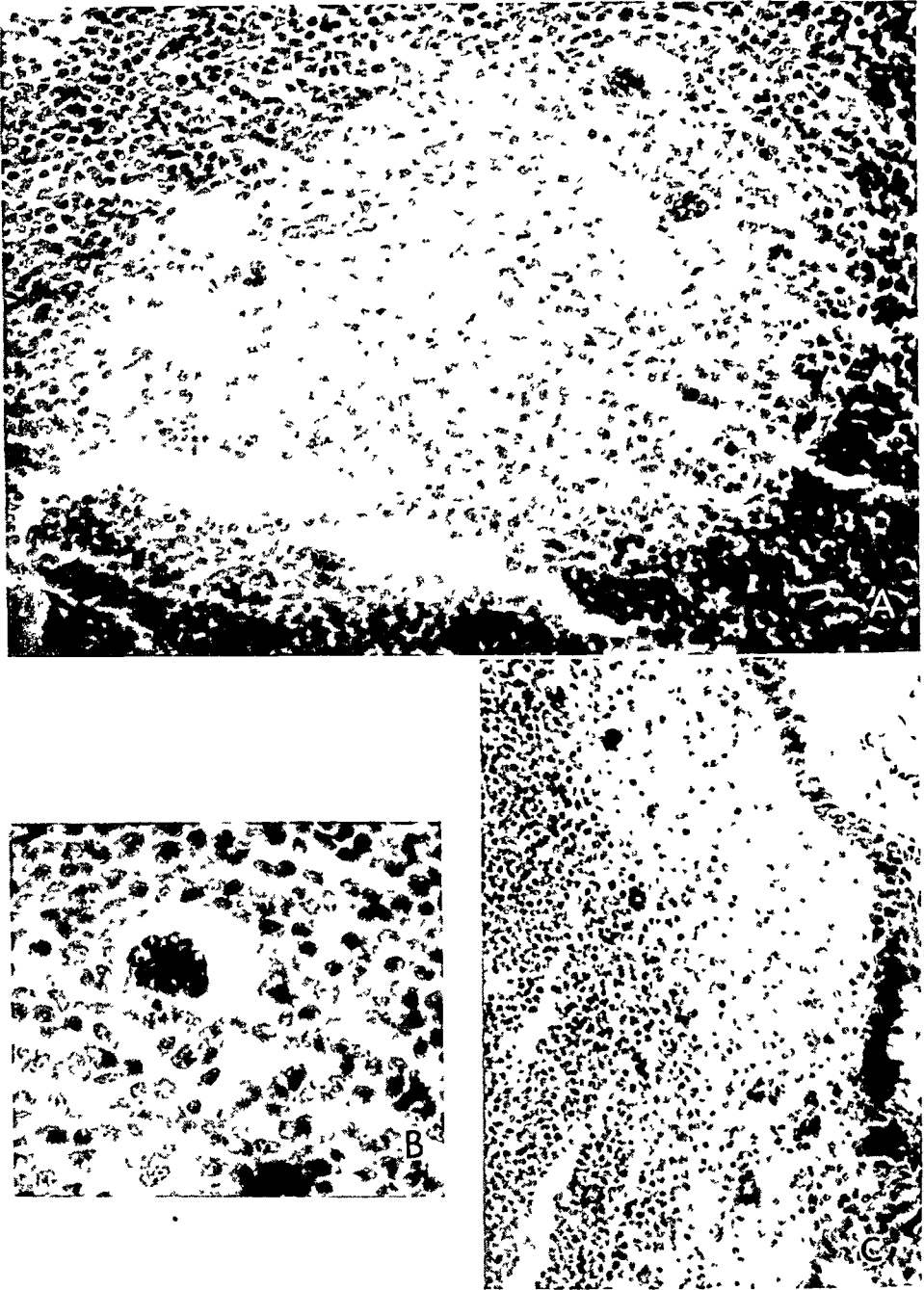


Fig. 1 (case 1).—*A*, photomicrograph showing a germinal center with three distinct giant cells,  $\times 220$ . *B*, photomicrograph of a high power magnification of a portion of the area shown in *A*. It includes the giant cell seen at the upper border of the germinal center. The clumped nuclei of the giant cell are surrounded by a wide zone of clear protoplasm. *C*, photomicrograph showing the giant cells in the subepithelial layers of the mucosa. They are mainly of the smaller compact variety;  $\times 120$ .

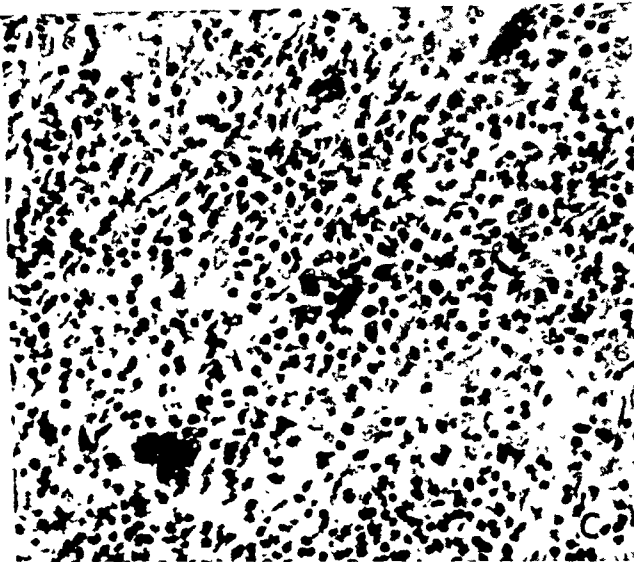
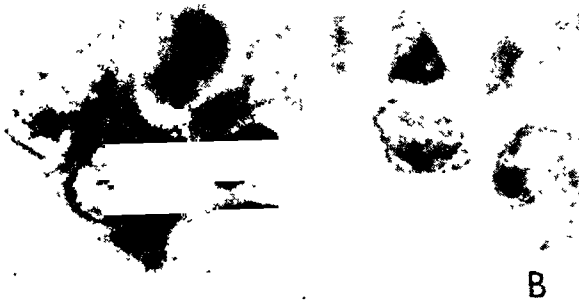
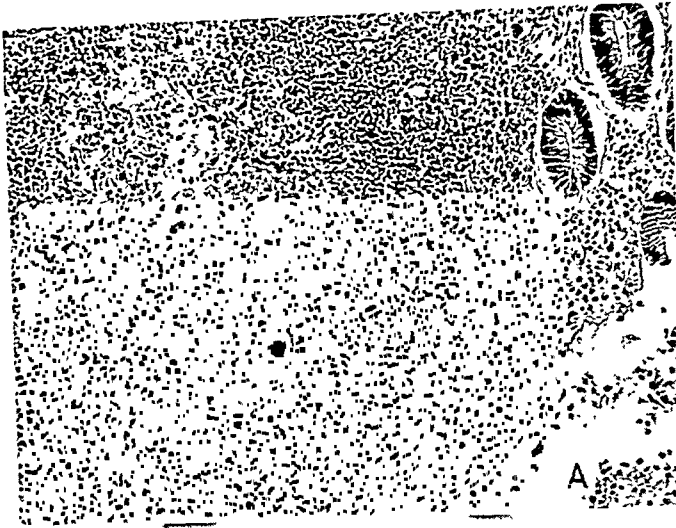


Fig. 2 (case 2).—*A*, photomicrograph showing a portion of the mucosa and a lymph follicle. The mucosa is diffusely infiltrated by neutrophilic leukocytes. A giant cell of the small compact variety is seen in the lower half of the follicle. The nuclei cannot be distinguished at this magnification;  $\times 100$ . *B*, a higher magnification of the giant cell seen in *A*. Here five distinct nuclei can be seen. The protoplasm in this small compact variety is very scanty;  $\times 1,800$ . *C*, photomicrograph showing a characteristic multinucleated giant cell in the left lower corner. The two other giant cells in the central portion and in the right upper corner are less distinct;  $\times 320$ .



ized in the right iliac region on the morning of admission. At about the same time with the onset of the abdominal pain there appeared a generalized cutaneous eruption. When examined on admission, he had Koplik's spots; his rash was macular and papular, covering the trunk and face. There were localized marked tenderness in the right iliac region and slight rigidity. Examination of the blood showed: hemoglobin, 80 per cent; red blood cells, 4,300,000; white blood cells, 12,000. The urine was normal. The diagnosis of acute appendicitis and measles was made. At the operation, which was performed two days after the appearance of the cutaneous eruption, more than four days after the onset of photophobia, and over five days after the respiratory symptoms had set in, a gangrenous retrocecal appendix was found. The child made an uneventful recovery.

Slides prepared from one block showed diffuse infiltration by neutrophilic leukocytes and fibrin. A diagnosis of diffuse suppurative appendicitis was made by Dr. Perry G. Melnik of the Pathological Laboratories of the Cook County Hospital. Dr. Richard H. Jaffé, pathologist of the Cook County Hospital, kindly permitted us to study the remaining portion of the appendix. Serial sections were prepared. Most sections showed a very intensive, diffuse infiltration of all the layers of the wall by neutrophilic leukocytes and by fibrin, and extensive areas of necrosis leading to destruction of the mucosa and to marked desquamation of the epithelial and subepithelial layers. The normal histologic structure was obscured by the inflammatory infiltration. In a fair number of the sections, small multinucleated cells were found in the lymph follicles and in the subepithelial layers, resembling the small, compact, multinucleated cells seen in our first case. They had only a small number of nuclei (about from 3 to 6) and a scanty amount of protoplasm. With the help of high magnification, the single nuclei could be distinguished (fig. 2 *B*). In a small portion of one block, large, typical multinucleated giant cells were found in a lymph follicle (fig. 2 *C*).

The very small number of the characteristic giant cells found in this appendix, despite a study of serial sections of almost the entire specimen, is of considerable interest. It confirms the observation of Herzberg,<sup>9</sup> who was able to find giant cells in his case also only in a very limited portion of the appendix. The possible explanations of the scarcity of the giant cells will be discussed later.

CASE 3.—A boy, aged 6, was admitted to the surgical department of the Michael Reese Hospital on May 28, 1932. His temperature had been elevated for the preceding two days, and for twenty-four hours he had been complaining of a sharp, continuous pain in the right iliac region. His previous history was irrelevant. When he was examined on admission, his temperature was 102.6 F.; his pulse rate, 140; his respiration rate, 40. The tonsils were enlarged and congested. No other changes were noted in the mucous membrane of the mouth. The cervical lymph glands were slightly swollen. There were tenderness and rigidity in the right iliac region. Examination of the blood showed: red blood cells, 4,400,000; white blood cells, 12,150; polymorphonuclear leukocytes, 82 per cent; lymphocytes, 18 per cent. In the urine, some acetone was found, but no other abnormalities. The diagnosis of acute appendicitis was made, and the child was operated on immediately. At the operation, an acutely inflamed appendix was found.

Dr. Otto Saphir, pathologist at the Michael Reese Hospital, examined the specimen. He found gross and microscopic evidences of acute diffuse suppurative appendicitis. Besides the inflammatory changes, there were numerous giant cells containing from 3 or 4 to 30 or more nuclei. The nuclei were closely packed with a narrow rim of free peripheral cytoplasm. The nuclear chromatin was abundant and

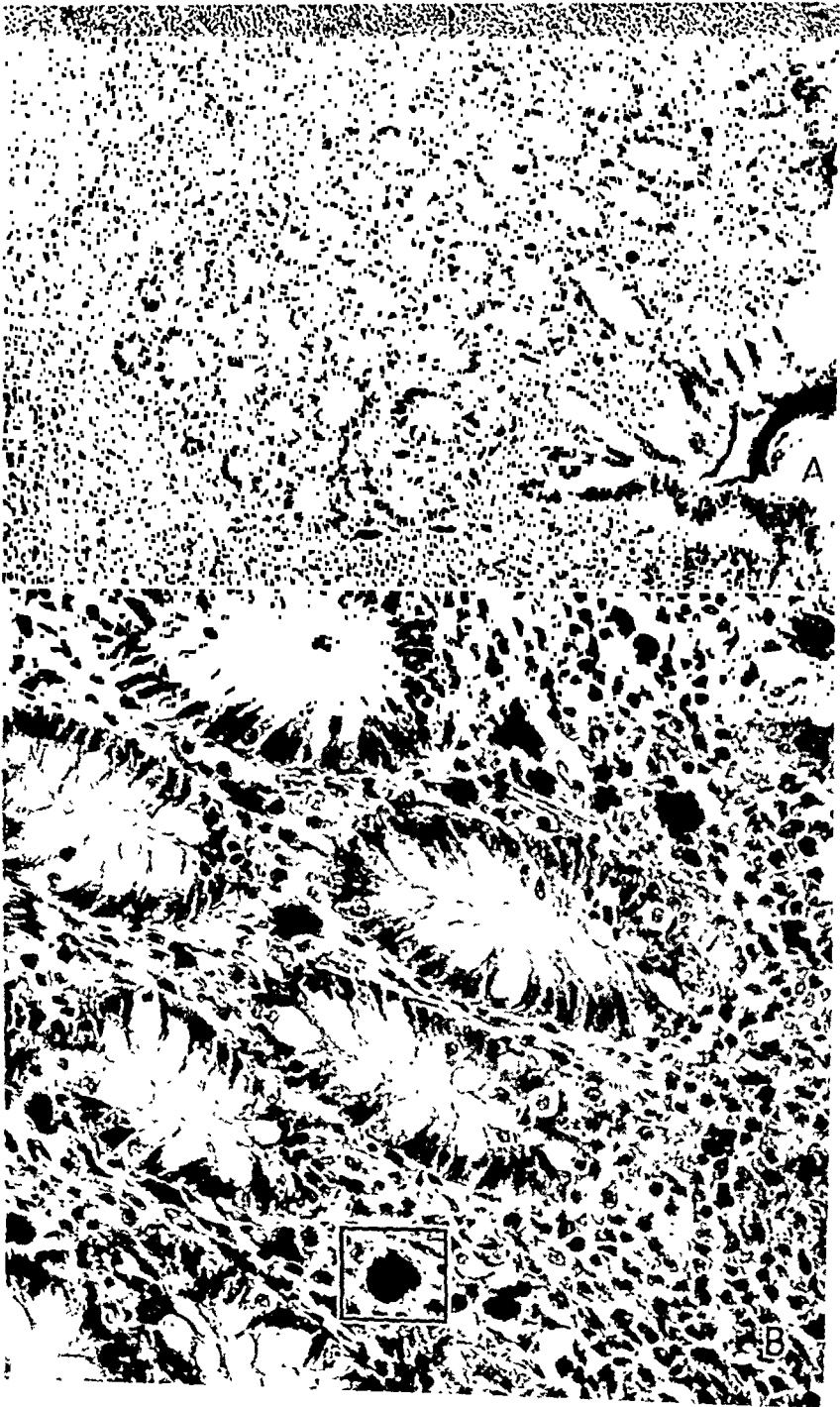


Fig. 3 (case 3).—*A*, low power photomicrograph showing large numbers of giant cells in the mucosa of the appendix;  $\times 80$ . *B*, a high power magnification of an area reproduced in *A*;  $\times 320$ .

coarsely trabeculated, strongly suggesting lymphocytic or plasma cell nuclei. The pathologic diagnosis was: "Acute diffuse suppurative appendicitis with giant cells in the appendix, characteristic of measles." Dr. Saphir's diagnosis was confirmed in the further course of the disease. Two days following the operation, a maculopapular rash appeared, and Koplik's spots were noted. There were 8,600 white blood cells. The diagnosis of measles was made. The child was isolated. The further course and recovery were uneventful. The child left the hospital on June 2, 1932.

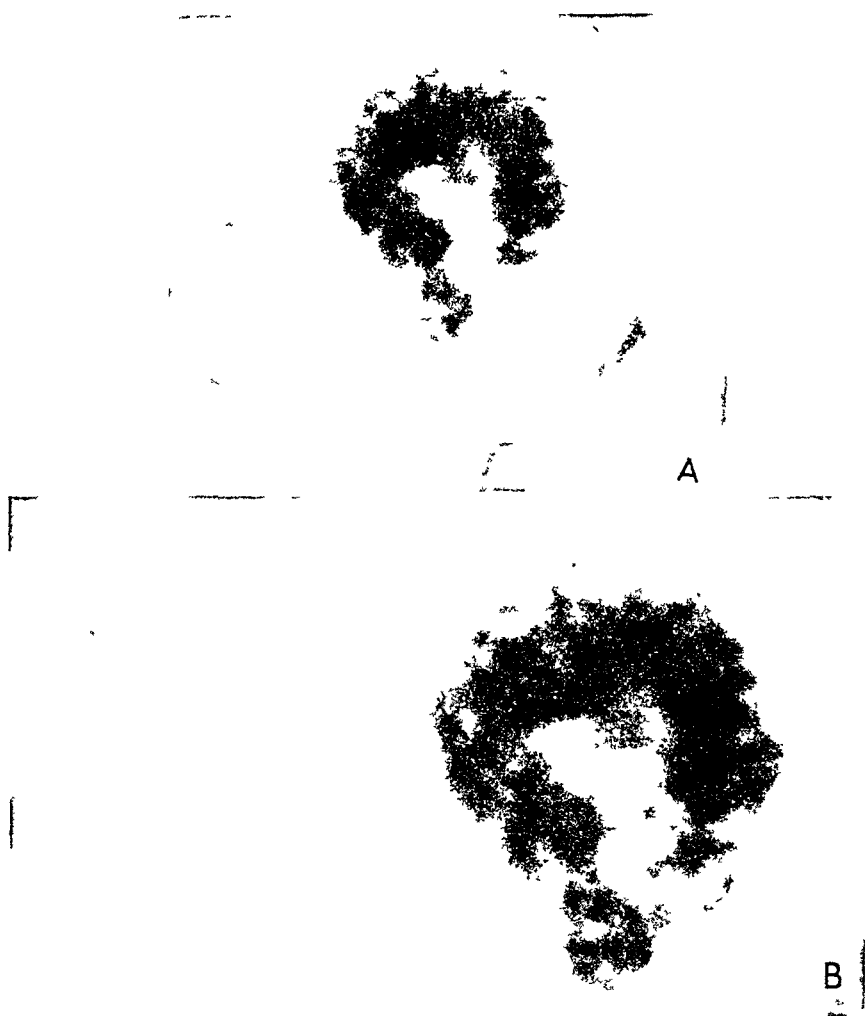


Fig. 4 (case 3).—*A*, a very high magnification of the giant cell seen in the lowermost portion of figure 3 *B*. The nuclei show a peripheral arrangement. The protoplasm is granular;  $\times 1,600$ . *B*, the same as *A*;  $\times 2,400$ .

Of the three blocks that were studied, two were the site of very intensive inflammatory changes, as described by Dr. Saphir, and in a large number of sections no giant cells could be detected. The third block, with only mild inflammatory changes, showed large numbers of the characteristic giant cells. In some sections up to 30 giant cells were counted in a low power field (fig. 3), but most fields had a lesser number. Only very few giant cells were seen in the lymph follicles; most of them were present in the mucosa, particularly in the subepithelial layers.

CASE 4.—A boy, 7 years old, was admitted to the Mount Sinai Hospital in the service of Dr. Philip L. Aries on May 19, 1932. About ten days previously Koplik's spots had appeared, and nine days previously a generalized cutaneous eruption had developed, diagnosed as measles. A younger child in the same family was recovering from measles. The disease in the case of the boy was mild. He had been out of bed for about four days when, on the evening before admission, he was seized with generalized abdominal pain, which soon became localized in the right iliac region. At the time of admission, there was marked tenderness with rigidity in the painful area. The temperature was 101 F. Examination of the blood showed 9,600 white blood cells, with 76 per cent polymorphonuclear leukocytes and 24 per cent lymphocytes. A diagnosis of acute appendicitis was made, and at operation an acutely inflamed, gangrenous retrocecal appendix was found. The further course and recovery were uneventful.

Histologic examination of the removed, grossly diseased appendix showed acute diffuse suppurative appendicitis and periappendicitis. Serial sections of the entire appendix were studied, but giant cells could not be found.

#### COMMENT

The abundance of the characteristic giant cells in the first and third cases of the series, their scarcity in the second and their complete absence in the fourth case suggest a relation between the presence of the giant cells in the lymph follicles and the subepithelial layers of the mucosa of the appendix and the stage of measles. In the two cases with the large numbers of giant cells the appendixes were removed two days before the appearance of the rash; in the second case, in which the giant cells were very scarce, the appendix was removed two days after the appearance of the rash, and in the fourth case, in which no giant cells were found, the appendix was removed nine days following the appearance of the cutaneous eruption. In the third case, the giant cells were found only in the parts of the appendix not markedly changed by the inflammatory process. While in the second case the relatively advanced stage of measles at which the appendix was removed was probably the main cause for the scarcity of the giant cells, the diffuse suppurative inflammatory process in the entire organ, which obscured the histologic relations and the cytologic details, may have been an additional factor.

#### SUMMARY

A histologic study was made of four appendixes, two of which were removed during the prodromal stage of measles, one during the course of the disease proper, and one after recovery. The presence of the characteristic giant cells in the mucosa and in the lymph follicles seems to have depended on the stage of the disease during which the organ was removed. The impression was gained that giant cells are rarely found in the portions of the appendix where profound diffuse inflammatory changes in the mucosa are present.

# PRIMARY IDIOPATHIC MUSCULAR HYPERTROPHY OF THE ESOPHAGUS WITH NARROWING OF THE LUMEN

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SAN FRANCISCO

Seven cases of primary idiopathic muscular hypertrophy of the esophagus with stenosis in which autopsy was performed have been recorded in medical literature. This condition is such a rarity, and the etiologic factors are so obscure that every new case reported may assist in elucidating its occurrence. I shall discuss briefly a case observed in the Stanford Medical Service at the San Francisco Hospital.

## REPORT OF CASE

*Clinical History.*—P. N., an American grocer, aged 69, was admitted to the San Francisco Hospital on Aug. 16, 1929, complaining of pain that had been present in both legs for seven months, weakness and inability to walk. During the past four years his habits had been irregular, he had consumed large quantities of alcoholic liquors, his appetite had gradually "dropped off," he had had "gas on the stomach," and his food "had not digested." He had had occasional gagging when he tried to eat, but no vomiting. For three weeks he had had diarrhea. One week before his entry into the hospital he had gone to bed and had not been able to get out since. Prior to this he had never been sick.

He appeared undernourished. The lungs were clear. The heart was normal. The peripheral blood vessels were markedly sclerosed. The blood pressure was 115 systolic and 85 diastolic. Marked wasting of the extremities and hyperesthesia of the legs were present. The reflexes were normal. Urinalysis revealed a light cloud of albumin and occasional granular casts. The blood was normal, except for a white blood cell count of 67,000, with 64 per cent polymorphonuclear leukocytes and 32 per cent lymphocytes. The Wassermann reaction was negative.

The patient became progressively weaker. Five days after his entry, administration of a barium meal was attempted, but roentgen examination was unsatisfactory because the meal gagged the patient. Examination at a later date was suggested in order to rule out possible carcinoma of the esophagus. Following the meal, the man declined rapidly and died two days later.

The clinical diagnosis was: chronic alcoholism, malnourishment and dehydration, generalized arteriosclerosis and possible esophageal cancer.

*Necropsy.*—Necropsy was performed on the day of death. The body was that of a strongly built, emaciated man, aged 70, of medium stature.

The anterior mediastinal tissue, the parietal pericardium, and the epicardium over the right side of the heart contained a large amount of fat. The heart was about

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three-fourths normal size. The aorta showed marked atheromatosis. The lungs showed old apical scars and moderate anthracosis.

The mucosa of the trachea was smooth and regular, showing no fistulas or other abnormalities. The trachea measured 7 cm. from the cricoid cartilage to the bifurcation and was 5 cm. in circumference.

The esophagus was in normal position. On cut section there was marked hypertrophy of the muscular wall in the lower third, involving chiefly the inner or circular layer and producing marked narrowing of the lumen in this region (fig. 1).



Fig. 1.—A cut section of esophagus, showing the marked hypertrophy of the muscular wall in the lower third.

The esophagus measured 19 cm. from the level of the cricoid cartilage to the cardiac orifice into the stomach. Ten centimeters below the cricoid cartilage the wall of the esophagus measured 3 mm. in thickness, and from this level distally there was a gradual, symmetrical and uniform increase in thickness until at the cardia the wall measured 12 mm. in width. Of this, the inner muscle layer measured 7 mm., and the outer, 3 mm. The mucous membrane was smooth and intact throughout, and showed no diverticula, fistulas or areas of dilatation.

A crater-like shallow diverticulum, 1 cm. in diameter, was found in the cecum. The mucosa of the colon throughout showed a number of small ulcers, which ranged in size up to 6 mm. in diameter.

The stomach contained a small amount of yellowish mucus. A small amount of residual barium was found in the fundus.

The liver was small, measuring 20 by 16 by 6.5 cm., and showed a moderate amount of fat on section.

The anatomic diagnosis was: arteriosclerosis, general, moderate; hypertrophy of the esophagus, with narrowing of the lumen; colitis, acute, ulcerative; alcoholism, chronic; diverticulum of the cecum; tuberculosis, pulmonary, apical, healed; fatty liver.

*Histologic Examination.*—The esophagus (1 to 2 cm. above the cardia) showed marked hypertrophy of the longitudinal and circular muscle layers, especially of the

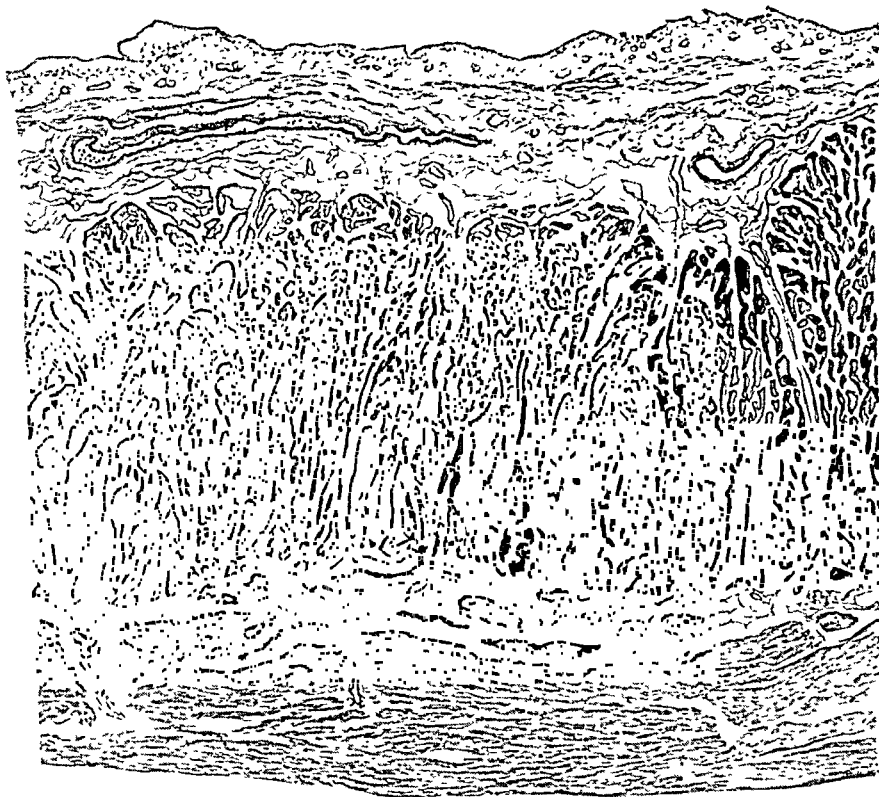


Fig. 2.—A microscopic section of the esophageal wall just above the cardia;  $\times 8$ .

latter. The mucosal epithelium was of stratified, squamous type and of normal thickness. Some of the superficial cells showed beginning keratinization.

A longitudinal section of the hypertrophied area of the esophagus showed that the circular layer was about three times the width of the longitudinal (fig. 2). No round cell infiltration or other abnormalities were noted. The epithelium was of normal thickness and appearance. The papillae showed no abnormalities. The nerve ganglions were normal. The surrounding blood vessels showed a slight thickening of their walls.

The cardia was normal.

The liver showed moderate congestion with beginning atrophy of the central cells and moderate fatty infiltration.

## SPECIAL FEATURES OF THE CASE

1. The age of the patient was 69 years; the sex, male.
2. There was no history of difficulty in swallowing, except for progressive failure of appetite during the past four years.
3. The heart was atrophied, and there were no enlarged mediastinal nodes or tumor masses that might have exerted pressure on the esophagus.
4. The esophagus was of normal length. Absence of diverticula, fistulas, etc., was demonstrated.
5. The hypertrophy was most marked in the circular muscle layer of the esophagus, beginning below the bifurcation of the trachea and becoming progressively greater, extending to the cardia.
6. The lumen of the area in which hypertrophy was present was narrowed.
7. There was no dilatation above the area in which hypertrophy and narrowing of the lumen occurred.
8. The cardiac orifice was normal, with no evidence of stricture or of obstruction.
9. There were no microscopic lesions in muscle, nerve ganglions, interstitial tissue and mucous membrane.

PATHOLOGY OF IDIOPATHIC MUSCULAR HYPERTROPHY  
OF THE ESOPHAGUS

In a review of the cases which have been reported in the literature and in conjunction with the case that I have described, I shall attempt to summarize briefly present knowledge concerning the occurrence and the nature of this type of esophageal hypertrophy. This condition may truly be rated among the pathologic curiosities. Owing to the vagueness or the absence of symptoms, it is difficult to make a diagnosis during life. For that reason all the known cases have been discovered at autopsy. In the seven cases previously reported the ages of the patients ranged from 39 to 68 years. Six of the patients were men; one, a woman.

Historically, this condition is discussed by the earlier authors, most of whom, however, had not seen actual cases.

Zenker and Ziemssen<sup>1</sup> up to 1878 had never observed a case of pure idiopathic muscular hypertrophy, although they conceded that such an independently occurring hypertrophy with stenosis was possible.

Förster<sup>2</sup> and Niemeyer<sup>3</sup> in 1865 stated that esophageal stenosis with muscular hypertrophy might occur, but that they believed it to be caused by "chronic catarrh."

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1. Zenker, F. A., and Ziemssen, H.: *Handbuch der speziellen Pathologie und Therapie*, Leipzig, F. C. W. Vogel, 1878.

2. Förster, A. J. T.: *Handbuch der pathologischen Anatomie*, Leipzig, L. Voss, 1863.

3. Niemeyer, F.: *Lehrbuch der speziellen Pathologie und Therapie*, Berlin, A. Hirschwald, 1865.



Birch-Hirschfeld<sup>4</sup> in 1887 wrote that in some cases of marked hypertrophy no reason for the stenosis was demonstrable.

Kitt<sup>5</sup> in 1895 mentioned the interesting occurrence of this condition in horses, namely, hypertrophy of the esophageal wall of as much as an inch in cross-section, converting the esophagus into a hard, thick, rigid tube. Interestingly, the condition involved chiefly the circular musculature.

Thus, in the older literature a few references to this condition can be found. However, these reports lack exhaustive descriptions, as well as accounts of microscopic examinations.

Recently, Henke and Lubarsch<sup>6</sup> included only two cases as well authenticated; these are the cases of Elliesen<sup>7</sup> and Ehlers.<sup>8</sup> For the period 1888 to 1930 I found described in the literature seven cases which I consider genuine and which were not associated with malignant change or with proximal dilatation. These are the cases of Pitt,<sup>9</sup> Rolleston,<sup>10</sup> Elliesen,<sup>7</sup> Ehlers,<sup>8</sup> Brücke,<sup>11</sup> Rake<sup>12</sup> and Goedel.<sup>13</sup>

#### ETIOLOGY OF HYPERTROPHY OF ESOPHAGEAL MUSCULATURE WITH NARROWING OF THE LUMEN BUT WITHOUT PROXIMAL DILATATION

Many etiologic and contributing factors in esophageal hypertrophy have been cited, such as: (a) simple overwork, due to the swallowing of large bites of food; (b) foci of nerve irritation, causing achalasia; (c) stenosis due to compression, i. e., by a hypertrophied heart, enlarged mediastinal nodes, an aortic aneurysm, etc.; (d) obstruction from within, obliteration stenosis, stenosis caused by carcinoma, annular hypertrophy; (e) stricture following erosion and chronic ulceration, and (f) chronic catarrh.

None of these many factors is common in the cases reviewed. Simple overwork and foci of irritation seem unimportant.

Compression stenosis can likewise be discarded, although its importance has been emphasized by several authors. Pitt<sup>9</sup> and Wilks and

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4. Birch-Hirschfeld, F. V.: *Lehrbuch der pathologischen Anatomie*, Leipzig, F. C. W. Vogel, 1887.

5. Kitt, Theodor: *Lehrbuch der pathologischen Anatomie*, Stuttgart, F. Enke, 1895.

6. Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 4, p. 105.

7. Elliesen: *Virchows Arch. f. path. Anat.* **172**:501, 1903.

8. Ehlers: *Virchows Arch. f. path. Anat.* **184**:512, 1907.

9. Pitt, G. N.: *Tr. Path. Soc. London* **39**:109, 1888.

10. Rolleston, D. D.: *Tr. Path. Soc. London* **50**:69, 1899.

11. Brücke, E. T.: *Virchows Arch. f. path. Anat.* **270**:880, 1928.

12. Rake, G. W.: *Guy's Hosp. Rep.* **166**:145, 1926.

13. Goedel, A.: *Wien. med. Wchnschr.* **79**:967, 1929.

Moxon<sup>14</sup> noted association of hypertrophy of the esophagus with that of the heart. Against compression by mediastinal masses are their absence in this series of cases and their common occurrence as compared with the incidence of the esophageal lesion.

Obstruction from within does not occur in this series. Greigh<sup>15</sup> pointed out that in cases with obstruction from within, the outer as well as the circular muscle layer is hypertrophied. He attempted to distinguish between the hypertrophy occurring subsequent to congenital stenosis and that in the cases of so-called idiopathic dilatation. In the former, the hypertrophy affects both longitudinal and circular layers; in the latter, only the circular.

Brücke<sup>11</sup> advanced the theory that the condition is equivalent to diffuse myoma, but this was denied by Goedel,<sup>13</sup> who said that it is wrong to speak of "diffuse myoma of the esophagus," since the change is concerned with a quantitative increase only, which in every respect is of regular growth.

Chronic catarrh was for a long time held to be associated with muscular hypertrophy of the esophagus, but this idea has been generally abandoned.

Niemeyer<sup>3</sup> stated that stenosis of the esophagus may at times depend on hypertrophy of the musculature and interstitial connective tissue. The mucosa is always simultaneously thickened with the increase in connective tissue and nonuniformly swollen.

Roe<sup>16</sup> reported a case in which there was an increase in the thickness of the esophageal wall in the vicinity of the tracheal bifurcation. Microscopically, the muscular fibrillae had become degenerated and had been replaced by a connective tissue formation. Therefore, the actual condition was pseudohypertrophy.

Goedel<sup>13</sup> inclined to the hypothesis of an embryonic anomaly,—of a primary congenital disturbance of growth in the sense of an inclination of the esophageal musculature to overgrowth. He believed that the "endogenous factor of the individual, inborn predisposition to increased growth," plays an important rôle in the case of all hypertrophies, even the acquired.

Elliesen,<sup>7</sup> having reviewed all the possibilities, found no satisfactory explanation, and therefore felt justified in saying that the condition appeared to be idiopathic, affecting chiefly the male sex, and occurring in middle or later life.

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14. Wilks and Moxon: *Tr. Path. Soc. London* **39**:107, 1888.

15. Greigh, D. M.: *Edinburgh M. J.* **26**:342, 1921.

16. Roe, J. O.: *New York M. J.* **53**:289, 1891.

## COMMENT

A clear distinction should be made between the cases discussed here and those with dilatation of the esophagus (esophagectasia), which are recognized not infrequently and are by no means uncommon. Although the two conditions are anatomically different and vastly apart in incidence, they have common developmental factors. One might even be inclined to propose the thesis that they are manifestations of the same process, dilatation occurring as a sequel to hypertrophy and stenosis. Symptoms as a rule do not arise until the dilatation has become well established.

Probably the most nearly correct interpretation is that the muscle hypertrophy occurs as the result of a completely compensated achalasia<sup>17</sup> of the esophagus. This hypothesis was first suggested by Dr. Brown Kelly and Prof. S. G. Shattock<sup>18</sup> in discussing Rolleston's case.<sup>10</sup> This would place the hypertrophy as one of the sequelae of "cardio-spasm."<sup>19</sup>

In the majority of cases of both conditions, that is, hypertrophy with narrowing of the lumen and ectasia, no lesions are found, the cause of the obstruction, if existent, being attributed to functional disturbances. In only one of the cases in this series<sup>12</sup> were microscopic lesions found. Sir Arthur Keith, who examined Rake's slides, noted that the large size of the ganglions present was similar to that found in ganglions near the pelvic-rectal sphincter in Hirschsprung's disease, and Hurst<sup>18</sup> expressed the belief that the latter condition is due to an achalasia of the pelvic-rectal junction. Hurst also expressed the belief that the majority of cases of esophageal achalasia are due to progressive organic disease involving Auerbach's plexus in the lower part of the esophagus. In Rake's case, the most advanced lesions were in the plexus below the hypertrophy, possibly leading to lack of coordination or spasm of the esophagus in the diseased portion. As a result, hypertrophy occurred proximally.

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17. Achalasia, failure to relax, defined by Hurst (Proc. Roy. Soc. Med. **12**:34, 1918) as a functional stenosis due to failure of the cardia to relax.

18. Shattock, S. G.: Proc. Roy. Soc. Med. **12**:95, 1918-1919. Hurst, A. F.: Proc. Roy. Soc. Med. **12**:34, 1918.

19. Cardiospasm, increased muscular tonicity or spasm of the musculature at the cardia. There is much discussion in the literature as to which condition is primary, cardiospasm or phrenospasm. The latter is a functional spasm of the musculature of the crura forming the margins of the hiatus, resulting in stenosis at the diaphragm—the stenosis, therefore, not being primarily esophageal (Jackson, Chevalier: Proc. Roy. Soc. Med. **12**:35, 1918-1919. Hill, G. W.: *ibid*, p. 66). For the purpose of this paper, however, it suffices to say that general ectasia of the thoracic gullet is secondary to primary obstruction of one or the other portions of the phrenocardiac segment of the esophagus; brought about by muscular malaction or overaction.

Hypertrophy occurs as long as compensation is maintained, then dilatation. This is analogous to what happens with the heart in hypertension, and with the urinary bladder in obstruction, and, in fact, is true of any tubular-muscular organ. Nerve lesions are not necessary, because there may be an inherent failure of the muscle to maintain compensation.

Those cases in which the hypertrophy commences above the cardia may be explained satisfactorily on the basis of the compensated achalasia. However, in those cases in which the hypertrophy continues into the cardia this explanation hardly seems satisfactory.

#### CONCLUSION

Cases of ectasia occur in all age groups, whereas those of hypertrophy with stenosis have been observed so far only in persons past 39 years of age and chiefly in the male sex. If the two were related to each other, hypertrophy should be found in the earlier age group. As Goedel<sup>13</sup> has written, "there seems to be some inherent, congenital factor which determines the occurrence and tendency for hypertrophy in 'idiopathic' as well as in acquired hypertrophies." The wide variation in the degree of the hypertrophy that occurs under similar given conditions is well known. As to the ultimate reasons for these inherent variations and for the achalias, one is still in the dark. It will suffice here to say, however, that these two factors, first, the congenital tendency to hypertrophy, and second, the development of a functional, completely compensated achalasia, may be of some importance in the muscular hypertrophy with narrowing of the lumen. In conclusion one has to acknowledge that no adequate explanation at present is available, although the hypothesis of a completely compensated achalasia may have some merit.

#### SUMMARY

A case of primary muscular hypertrophy of the esophagus affecting chiefly the circular layer, and accompanied by narrowing of the lumen, has been reported. The literature has been reviewed and searched for reports of similar cases, and several have been found. The views pertaining to the etiology of muscular hypertrophy with stenosis have been presented. The relation of this condition to ectasia of the esophagus has been discussed. The condition must still be considered as "idiopathic" until more is known of possible congenital tendencies to excessive development, and of the relation of the condition to achalasia of the esophagus.

# INFLUENCE OF LIVER EXTRACT AND ACUTE INFECTION ON THE RETICULOCYTES AND BONE MARROW OF PIGEONS

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In their first series of patients with pernicious anemia who were treated by liver therapy, Minot and Murphy<sup>1</sup> noted that infection inhibited the prompt reticulocyte response and the general improvement ordinarily obtained. This observation has subsequently been confirmed by many other investigators.<sup>2</sup> The exact mechanism, however, of the action on the bone marrow and the blood of the substance effective in pernicious anemia, as well as of the neutralizing effect of infection on the reticulocyte response, is still obscure.

The experimental approach to the question of the mechanism of the action of the principle effective in pernicious anemia has yielded little positive information. The potent material does not significantly influence the peripheral blood of normal rats,<sup>3</sup> rabbits and dogs.<sup>4</sup> Neither in anemias experimentally produced in mammals has any significant effect been obtained which could be ascribed to the action of the principle effective in pernicious anemia.<sup>5</sup> However, pigeons seem to be peculiarly sensitive to the potent material, and it has been shown<sup>6</sup> that relatively pure liver extracts injected intravenously, as well as commercial liver extract no. 343 (N. N. R.) fed by mouth, cause a typical reticulocyte response in normal pigeons. It was also noted that one bird that failed to show an increase in reticulocytes had a severely ulcerated mouth. It

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The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

1. Minot, G. R., and Murphy, W. P.: *J. A. M. A.* **87**:470, 1926.

2. (a) Vaughan, J. M.: *Quart. J. Med.* **23**:90, 1930. (b) Davidson, L. S. P., and Gulland, G. L.: *Pernicious Anaemia*, London, Henry Kimpton, 1930.

3. Vaughan, J. M., and Muller, G. L.: *J. Clin. Investigation* **11**:129, 1932.

4. Adlersberg, D., and Gottsegen, G.: *Arch. f. exper. Path. u. Pharmacol.* **142**:323, 1929.

5. Vedder, A.: *Nederl. tijdschr. v. geneesk.* **72**:4411, 1928. Vedder A.: *Ergebn. d. inn. Med. u. Kinderh.* **38**:272, 1930. Orban, M.: *Monatschr. f. Kinderh.* **42**:402, 1929. Robscheit-Robbins, F. S.: *Physiol. Rev.* **9**:666, 1929. Davidson and Gulland.<sup>2b</sup>

6. Vaughan, J. M.; Muller, G. L., and Zetzel, L.: *Brit. J. Exper. Path.* **11**:456, 1930.

therefore seemed as if sepsis, interfering with the response to the principle effective in pernicious anemia in man, might also play the same rôle in pigeons.

To elucidate further the action of liver extract on the red blood cells in the bone marrow, and to obtain some possible explanation for the failure of an adequate reticulocyte response in patients with pernicious anemia who were suffering from infections, a series of experiments has been carried out with the pigeon as the experimental animal. This paper deals with influence of acute infection and of administration of liver extract on the reticulocytes in the peripheral blood and on the histologic changes occurring in the bone marrow of pigeons.

#### METHODS

Healthy adult pigeons were used throughout. They were kept under standard laboratory conditions in individual cages and supplied daily with fresh water and a food mixture obtained in the market under the name of "pigeon feed." Grit was also supplied. On this regimen, untreated adult birds maintain their weight and general appearance of well-being indefinitely. All the animals were allowed to become stabilized as to weight and composition of the blood before the experiment was begun. These precautions are necessary because it has been found in this laboratory that young birds, in some instances, maintain a high reticulocyte count up to 6 months of age, and pigeons received in poor condition from the market respond with a reticulocyte rise when put on an ample diet.

The total number of red blood cells were counted and the hemoglobin content was determined at frequent intervals, and a smear of blood was taken almost daily for a count of the reticulocytes. At various stages, tissues from the liver, spleen and bone marrow were obtained by autopsy. The character of the tissues fixed in Zenker's fluid and stained with eosin and methylene blue (methylthionine chloride, U. S. P.), was correlated with the state of the peripheral blood. The radial bone marrow was used for comparisons, because this marrow in most healthy adult birds is mainly fatty, and increased hyperplasia and extension of the marrow can therefore be determined with ease. In a small number of animals, intravital puncture of the bone marrow was performed under local anesthesia before the infection or the administration of liver extract, in an attempt to correlate the changes of the bone marrow before and after the experimental procedure in the same animal. So as not to introduce an extraneous factor, only birds with minimal bleeding at the biopsy were employed.

In all, ninety-six pigeons were used. They were divided into three groups, which were given: (1) *Staphylococcus aureus* infection intramuscularly, (2) liver extract no. 343 (N. N. R.) by mouth, and (3) both infection and liver extract.

#### 1. EFFECT OF INFECTION ON THE RETICULOCYTES AND THE BONE MARROW

The organism used was obtained from a fatal case of *Staph. aureus* septicemia in man, and only this one strain was employed. Sixteen hour broth cultures were injected into the pectoral muscle in doses of from 0.5 to 1 cc. As a rule, 0.75 cc. was employed, as 0.5 cc. did not call forth striking changes, and 1 cc. doses were often fatal. In all,

thirty-four pigeons were used in this group, ten of which died in from twenty-four to forty-eight hours after the injection—a mortality of 29.4 per cent. In the case of a few animals dying from the infection and killed in the agonal stage, the organism was recovered from the heart's blood in pure culture. In sixteen birds the temperature was taken daily, and a definite reaction to the infection, manifested by fever, was observed. The white blood cells responded to the pyogenic infection with leukocytosis, a reaction similar to that found in most animals and in man. Here the attention, however, has been focused mainly on two features, namely, the action of the infection on the reticulocytes in the peripheral blood and the simultaneous reaction of the bone marrow.

The normal reticulocyte count of healthy *adult* pigeons under the standard conditions in this laboratory usually has been from 10 to 12 per cent. After infection had been introduced, there was a decrease in the percentage of reticulocytes in the peripheral blood. This decrease seemed to be commensurable with the severity of the infection, the lowest percentage of reticulocytes being obtained between the third and fifth days of the infection. At about the eighth to the tenth day of the infection, there was, however, an increase in the percentage of reticulocytes in the peripheral blood above normal values, the highest point being reached between the twelfth and seventeenth days with values ranging between 25 and 35 per cent. Then the percentage of reticulocytes decreased gradually until a normal level had been established. This reaction of the reticulocytes to infection is illustrated in the first part of the curve shown in figure 1. The reason for this outpouring of young reticulocytes in the peripheral blood became clear when bone marrows were examined at various stages after infection.

As has been pointed out, the normal radial bone marrow in the adult pigeon is mainly fatty with some cellularity at the edges. Occasionally the entire marrow is hyperplastic. This condition is usually associated in the healthy adult bird with a partial or total replacement with cancellous bone of the normally hyperplastic femoral bone marrow. To exclude this factor the femoral bone marrow was examined in every instance. Under ordinary conditions, however, increased activity and extension of the hematopoietic tissue can be easily discerned in the radial marrow. Throughout the comment, the nomenclature of Sabin<sup>7</sup> has been employed, and in evaluating any changes in the bone marrow both quantitative and qualitative aspects have been considered.

In the normal bone marrow of pigeons, the centers of red blood cell production consist of a mixture of cells ranging from megaloblasts to mature red blood cells. A considerable proportion of the cells are

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7. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: *Contrib. Embryol.* 16:125, 1925.

found to be in the megaloblastic and early erythroblastic stage, and considerable activity is present, as evidenced by mitotic figures. These centers of erythrocytic production in pigeons are definitely circumscribed, while the white blood cells present, mainly in the form of myelocytes or adult leukocytes, are scattered in between without any definite arrangement. Typical pictures of radial bone marrows from normal pigeons are illustrated in figure 2, *A* and *B*, showing various degrees of cellularity.

Twenty-four hours after a bird had been infected, the bone marrow had become actively *stimulated*. Between the fat cells appeared many

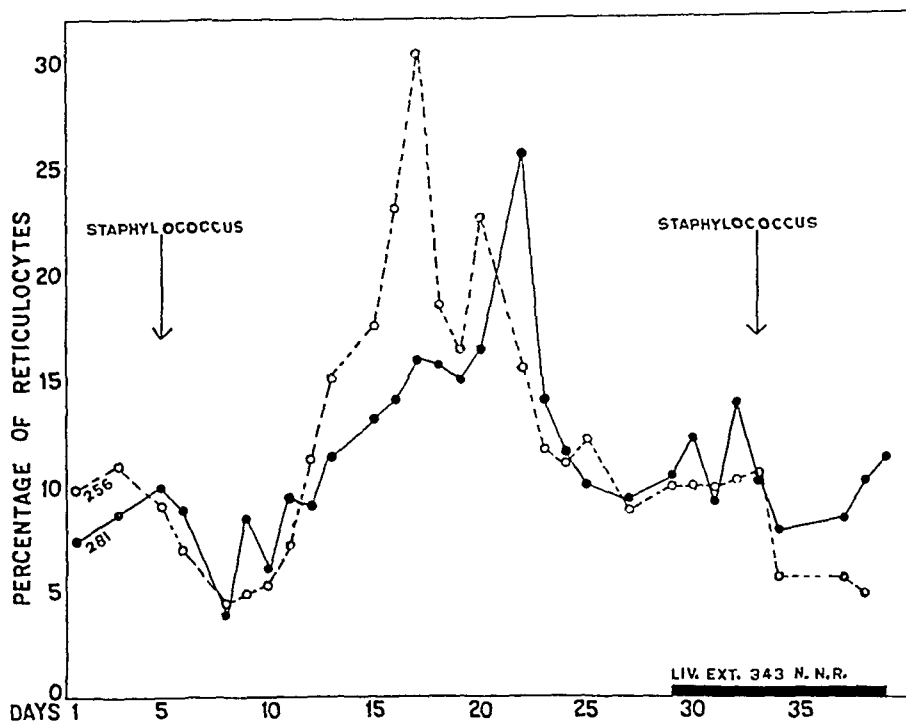


Fig. 1.—Reticulocytes of two pigeons (256 and 281) infected by injection of 1 cc. of a sixteen hour broth culture of *Staph. aureus*. Subsequently liver extract no. 343 (N. N. R.) was given by mouth, and the birds were reinfected.

immature cells belonging to both the red and the white blood cell series. The blood vessels were dilated. After forty-eight hours, the blood vessels remained engorged, and the hyperplasia was increased, with both the red and the white blood cells participating. The erythrocytic centers were distinguished in this early stimulated marrow by their situation in intersinusoidal capillaries, and by their more intense staining with methylene blue, as well as by nuclear and protoplasmic characteristics. The architecture of the bone marrows, twenty-four and forty-eight hours after infection, is illustrated in figure 2, *C* and *D*. With the oil immersion lens, mitotic figures were seen everywhere



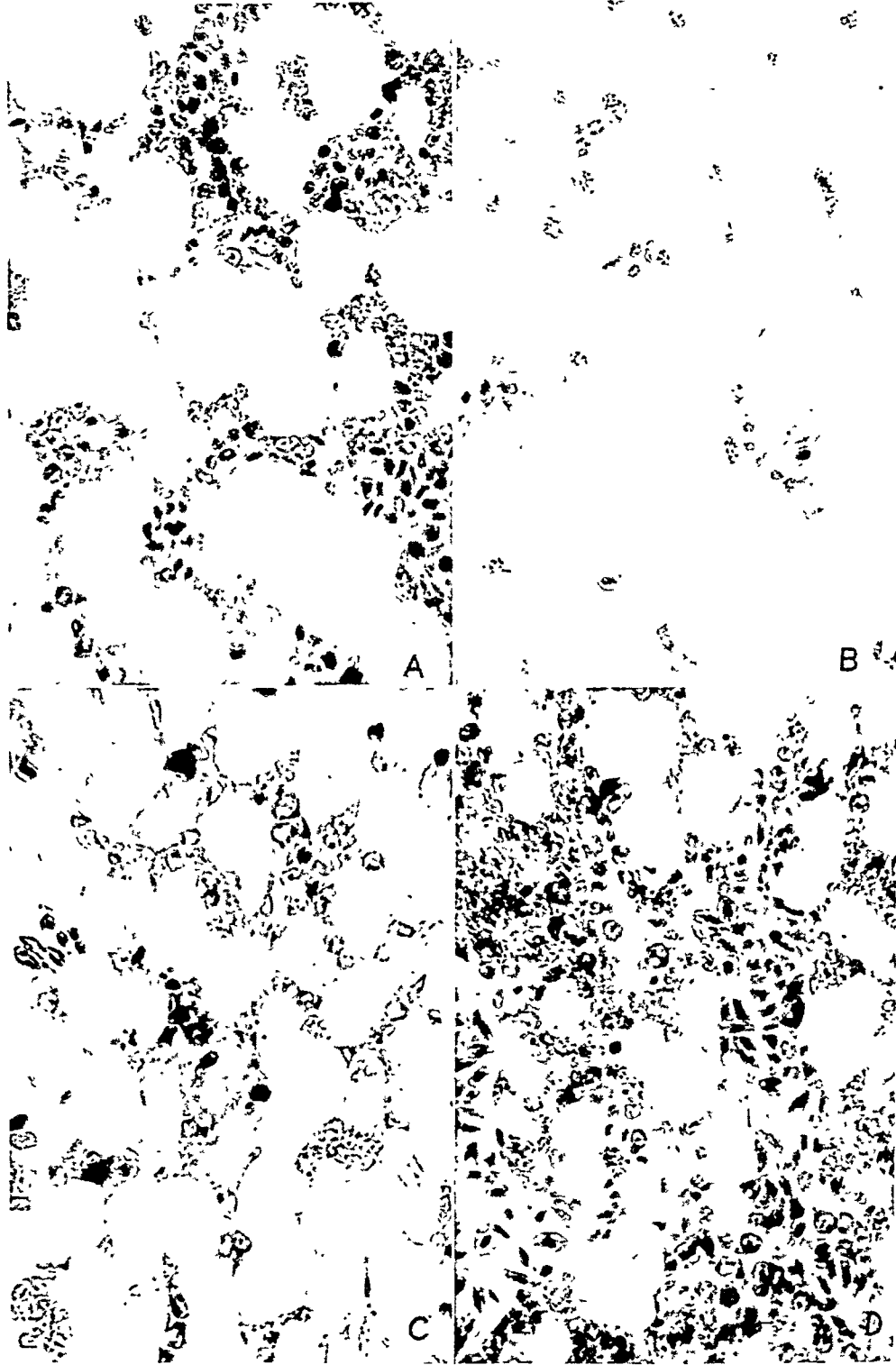
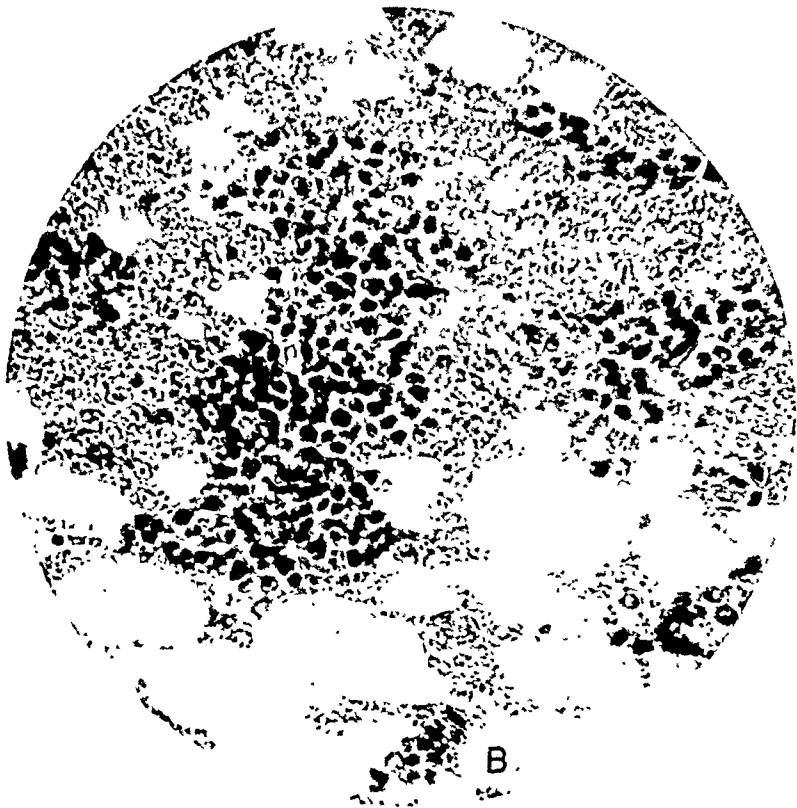


Fig. 2.—Appearance of marrow: *A*, moderately hyperplastic radial marrow of a normal pigeon with centers of production of red blood cells containing many megaloblasts;  $\times 500$ . *B*, fatty radial marrow of a normal pigeon;  $\times 500$ . *C*, marrow of a pigeon twenty-four hours after the pigeon had been infected by an intramuscular injection of *Staph. aureus*. It shows active stimulation of both white and red blood cells and hyperemia;  $\times 500$ . *D*, marrow of a pigeon after the pigeon had been infected with *Staph. aureus* for forty-eight hours. Note the increased hyperplasia of both white and red blood cells and the dilated vessels;  $\times 500$ .



A



B

Fig. 3.—Appearance of marrow: *A*, specimen shown in figure 2 *D*;  $\times 1,500$ . Note the megaloblasts with mitotic figures. *B*, radial marrow of a pigeon after the pigeon had been infected with *Staph. aureus* for five days. There is solid hyperplasia of both red and white blood cells;  $\times 500$ .

among the erythrocytic as well as the myelocytic series. It is of interest to note that mitosis occurred at the edges of fat cells, where usually, in the normal bird, only the elongated nuclei of endothelial cells are present (fig. 3 *A*).

A progressive increase in cellularity was seen on the third and fourth days, and after an interval of five days the cellularity of the radial marrow had increased tremendously. The islands of red blood cells were large and consisted of quite immature cells around the periphery, while in the center some practically mature cells were present. The character of such a bone marrow is shown in figure 3 *B*. On the seventh day a hyperplasia was seen quantitatively identical with that on the fifth day, with, however, some qualitative changes. The reaction of white blood cells had subsided, while the centers of production of red blood cells were even larger and more prominent with many immature cells still present. The bone marrow apparently was rapidly becoming ready to discharge the excess of red blood cells, and evidence of this discharge was obtained by the outpouring of reticulocytes in the peripheral blood (fig. 1). Subsequently there was a return of the peripheral blood and bone marrow to a normal histologic appearance. No reaction was observed in the peripheral blood or in the bone marrow after intramuscular injection of 1 cc. of sterile broth.

From the foregoing observations one may be justified in concluding that acute infection causes an active stimulation of both the white and the red blood cell series of the pigeon's bone marrow. This response of the erythrocytes later takes expression in an outpouring of reticulocytes, apparently commensurable with the number of red blood cells that have been produced in the marrow. The phase of decreased percentage of reticulocytes in the peripheral blood corresponds to the rapid growth of the megaloblasts and early erythrocytes in the bone marrow.

## 2. EFFECT OF ADMINISTRATION OF LIVER EXTRACT NO. 343 (N. N. R.) ON THE RETICULOCYTES AND THE BONE MARROW

To this group of pigeons, thirty in all, 2 cc. of a 50 per cent solution of liver extract was administered daily by means of a stomach tube, after a preliminary control period.

Previous observations<sup>6</sup> on the response of the reticulocytes to the administration of substances effective in pernicious anemia were verified. In most instances, the count of reticulocytes at the peak of their initial response in the pigeons under the laboratory conditions employed was in the vicinity of 20 per cent. This same magnitude of response was obtained also with comparatively pure liver extracts.<sup>6</sup>

The bone marrow of a pigeon that had had liver extract for four days by mouth is illustrated in figure 4 *A*. At this time one would

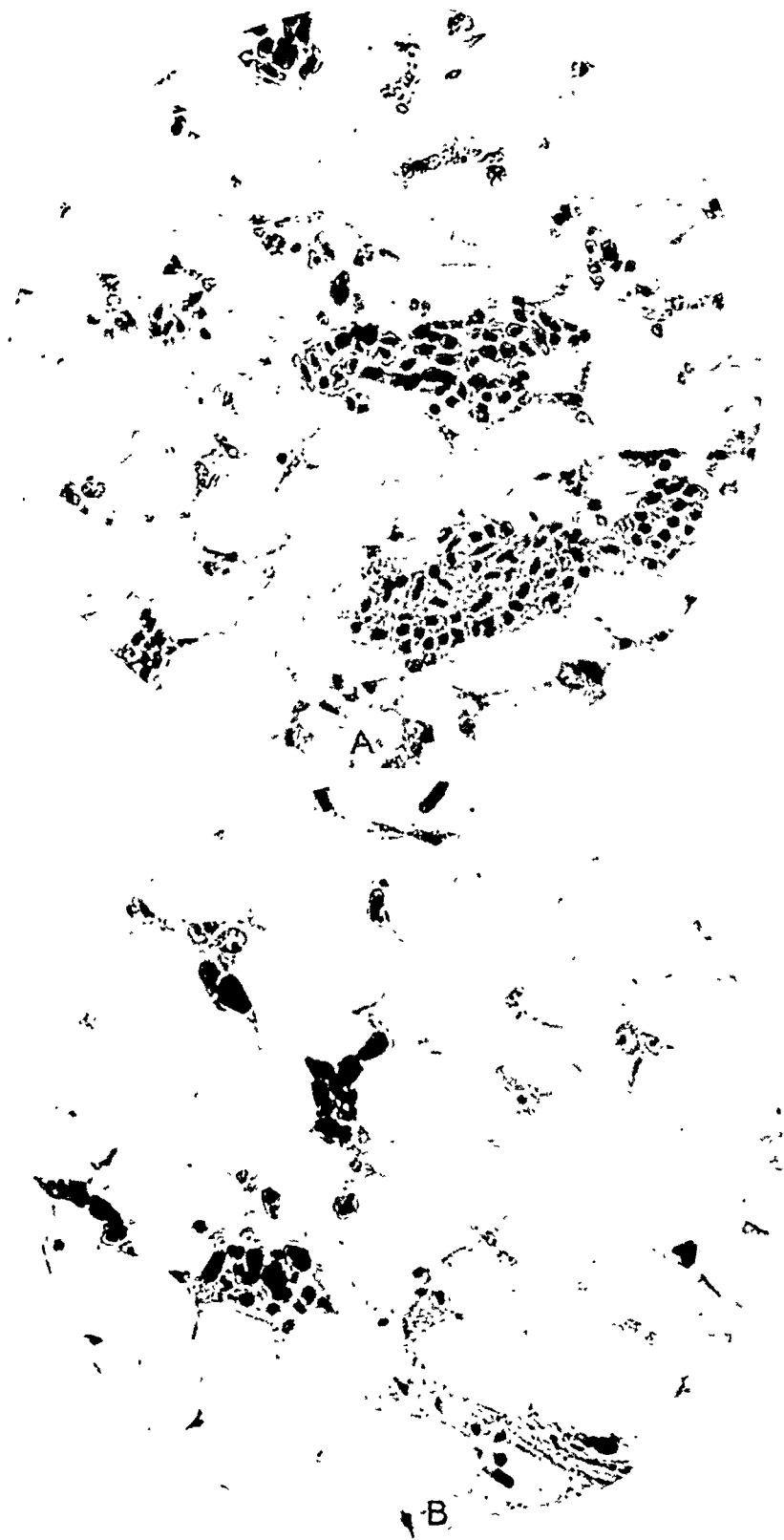


Fig. 4.—Appearance of marrow: *A*, radial marrow of a pigeon that had received liver extract daily for four days. Note the changed character of the two centers of production of red blood cells in the lower right sector. Practically all the cells are normoblasts;  $\times 500$ . *B*, radial marrow of a pigeon that had received liver extract for seven days. Note the aplastic character of the radial marrow obtained when reticulocytes were 20 per cent in the peripheral blood;  $\times 500$ .



Fig. 5.—Appearance of marrow: *A*, normal center of production of erythrocytes showing megaloblasts, erythroblasts, normoblasts and mitotic figures;  $\times 1,500$ . *B*, center of production of erythrocytes from a pigeon that had received liver extract by mouth daily for sixteen days. Note the absence of megaloblasts. A few erythroblasts are present, but the majority of the cells are in the normoblastic stage;  $\times 1,500$ .

expect an increase and extension of the erythropoietic tissue to herald the reticulocyte increase in the peripheral blood. However, no stimulation was discernible from either the quantitative or the qualitative point of view. There seemed to be rather a diminution of megalo-blasts, or immature red blood cells, and the centers of erythrocytic production consisted mainly of mature or nearly mature red blood cells.

Figure 4 *B* shows the radial bone marrow of a bird on the seventh day after the commencement of administration of liver extract. No

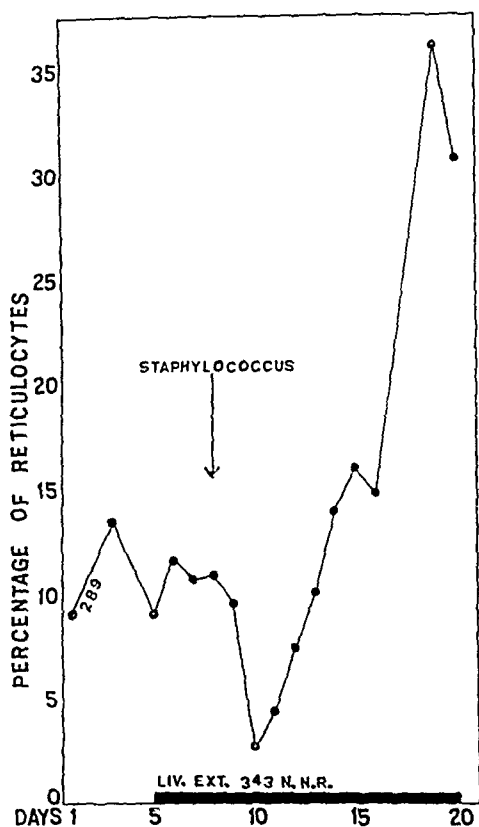


Fig. 6.—Reticulocyte response of a pigeon (289) given liver extract no. 343 (N. N. R.) and infected with an injection of a broth culture of *Staph. aureus*. Note the decrease of reticulocytes and the subsequent increase to 35 per cent.

comment beyond the descriptive legend is necessary. Identical results were obtained at intermediate periods up to sixteen days, the interval of time covered in these experiments. In no instance was there a stimulation of the erythroblastic tissue; rather a qualitative change in the islands of red blood cells was observed, with a diminution of megalo-blasts and mitotic figures, as far as could be determined by counts on fixed and stained specimens. Quantitatively there appeared to be a decrease of erythropoietic tissue.

The contrast between the composition of a comparatively large center of production of red blood cells in the femoral bone marrow in a healthy bird without any treatment and that in a pigeon receiving liver extract by mouth daily for sixteen days is illustrated by figure 5 *A* and *B*. The absence or numerical diminution of megaloblasts in the bird receiving liver extract and the absence of evidences of activity, such as mitotic figures, were, to say the least, striking. Megaloblasts and mitotic figures are always present in the bone marrow of normal pigeons. Liver extract apparently transforms the pigeon's bone marrow from a megaloblastic to a late erythroblastic and normoblastic one, at least temporarily. This is accompanied by an outpouring of reticulocytes in the peripheral blood probably due to a simultaneous development of a large number of red cells of approximately the same age. At this stage, the reticulocyte response is not accompanied by increased activity in the sinusoidal endothelium to replace the lost cells; rather inactivity is present, as far as can be judged from the histologic picture of the bone marrow.

### 3. INFLUENCE OF LIVER EXTRACT NO. 343 (N. N. R.) AND INFECTION ON THE RETICULOCYTES AND THE BONE MARROW

In this group of thirty-two pigeons, 2 cc. of liver extract no. 343 (N. N. R.) was given daily for from three to seven days before the bird was infected, and its administration was continued until the animal was killed. Of these, three were infected with the organism intramuscularly on the same day that the administration of liver extract was begun. This procedure was discontinued, since the infection under these circumstances became altogether the predominating feature. Of the thirty-two birds infected, twelve died within forty-eight hours—a mortality of 37.5 per cent.

In birds infected before there was any appreciable rise in reticulocytes from the administration of liver extract, the reticulocyte curve obtained could not be distinguished from the one resulting from infection alone. There was the initial decrease of reticulocytes below the normal level and then there was an increase up to from 25 to 35 per cent. This is illustrated in figures 1 and 6.

When a reticulocyte response to the liver extract had been obtained, and the birds were infected at the presumable height of this response, the reticulocyte curve descended precipitously in spite of the daily administration of liver extract (fig. 7).

Representative bone marrows at various stages showed a somewhat less definite picture than those obtained with either infection or liver extract alone. In all instances, however, the infection predominated.

The bone marrow of a bird infected on the seventh day of the administration of liver extract and killed four days later is illustrated in figure 8 *A* and *B*. The reticulocyte count at the time of infection was 17.6 per cent, and on the fourth day of infection, at which time the bird appeared acutely ill, it was 3.7 per cent. The bone marrow at that time showed increased cellularity, constituted of both white and red blood cells, and extensive stimulation, with many mitotic figures. It seemed as if the hyperplasia was not so far advanced as in those animals subjected to infection alone, but this may have been due to an individual difference in this animal.

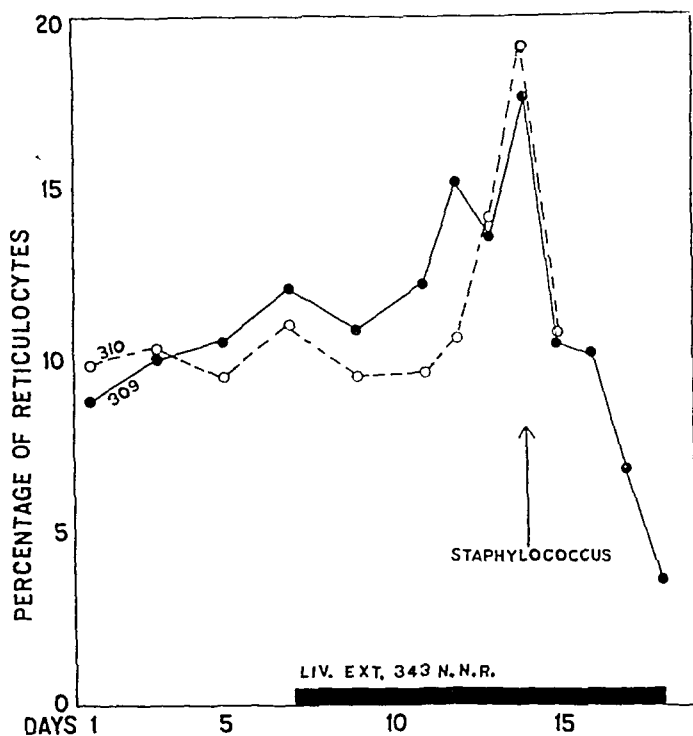


Fig. 7.—Reticulocyte response of two pigeons (309 and 310) to liver extract no. 343 (N. N. R.) and superimposed infection. The reticulocyte response to liver extract was interrupted by the infection, and subsequently the reticulocytes fell below a normal level.

The bone marrow of one of the birds, the reticulocyte counts of which are recorded in figure 1, is illustrated in figure 9. This bird had liver extract for a total of nine days and infection for five days. As may be seen from figure 1, this pigeon had been infected one month earlier and had recovered. It was thought that some resistance to infection might have developed, and therefore a somewhat larger dose of the broth culture was injected (1.5 cc.) the second time. The bone marrow showed intense hyperplasia of white blood cells. The centers of production of red blood cells had not participated to the same extent



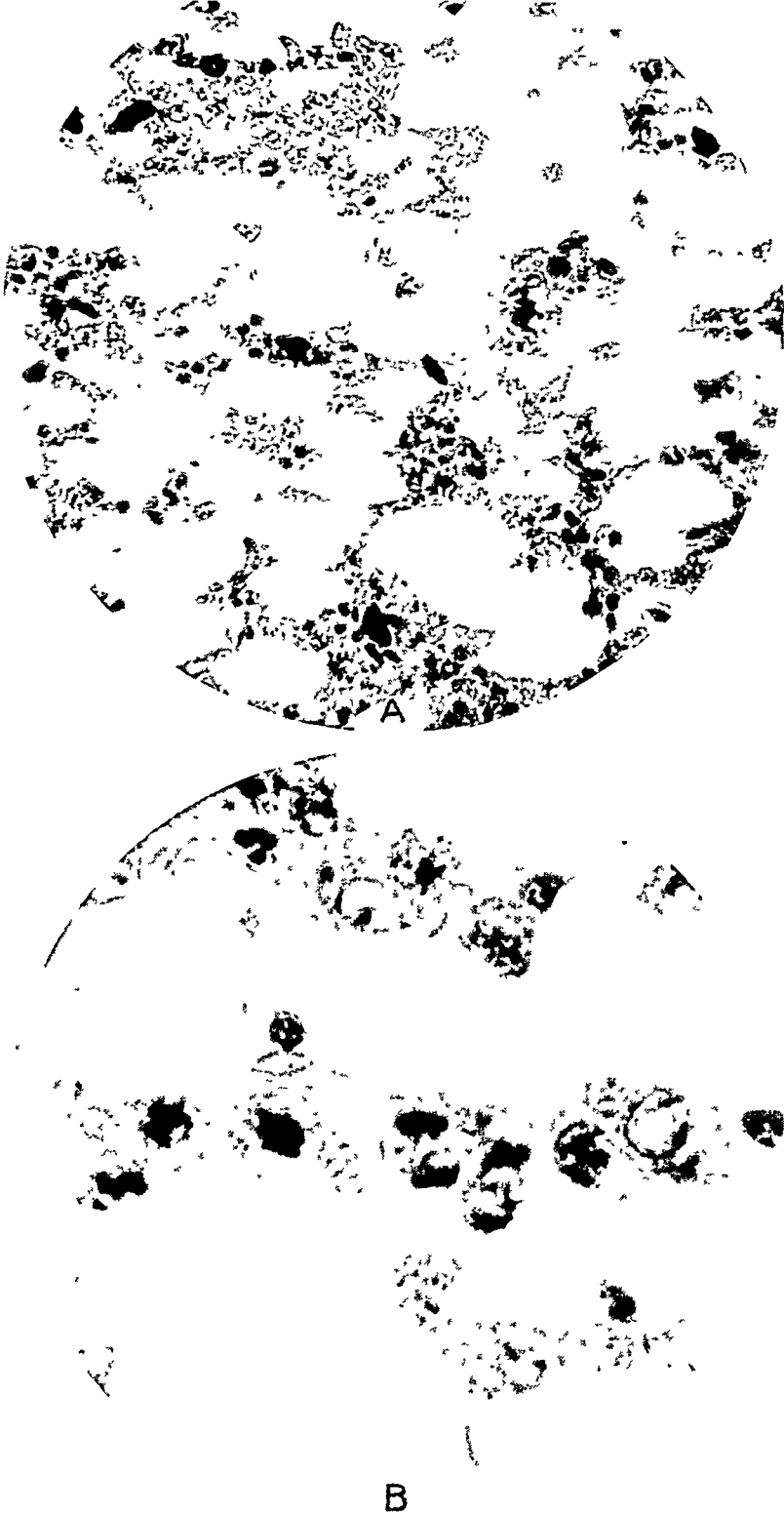


Fig. 8.—Appearance of marrow: *A*, marrow of a pigeon treated with liver extract for seven days, with an infection that had been present for four days. The bird was infected when the reticulocytes were 17.6 per cent. When the bone marrow was obtained the reticulocytes were 3.7 per cent. Active stimulation of both red and white blood cells was present, but this reaction was somewhat less advanced than in animals which had been infected for four days but which had not been given liver extract;  $\times 500$ . *B*, specimen shown in *A* ( $\times 1,500$ ) showing active mitosis in a row of megaloblasts.



Fig. 9.—Appearance of marrow: *A*, marrow of a pigeon (fig. 1) which had been given liver extract for nine days, and which had been infected for five days. The infection resulted from an injection of  $1\frac{1}{2}$  cc. of a broth culture of *Staph. aureus*. Note the solid hyperplasia mainly of white blood cells with small centers of production of erythrocytes, which consisted of either practically mature cells or young megaloblasts in active mitosis;  $\times 500$ . *B*, same specimen as shown in *A* ( $\times 1,500$ ). Note the small center of production of megaloblasts with mitotic figures. *C*, same specimen as shown in *A*. Note the center of production of red blood cells consisting mainly of normoblasts shrunk from the edges of the sinusoidal capillary;  $\times 1,500$ .

(fig. 3 *B*). They were small, containing mainly mature cells, which had shrunk from the edges of the intersinusoidal capillaries, which, as a rule, are lined with megaloblasts. However, here and there were small centers consisting entirely of megaloblasts in active mitosis. It seemed as if two influences had been at work, namely, the liver extract, which had influenced the majority of the red blood cells present before

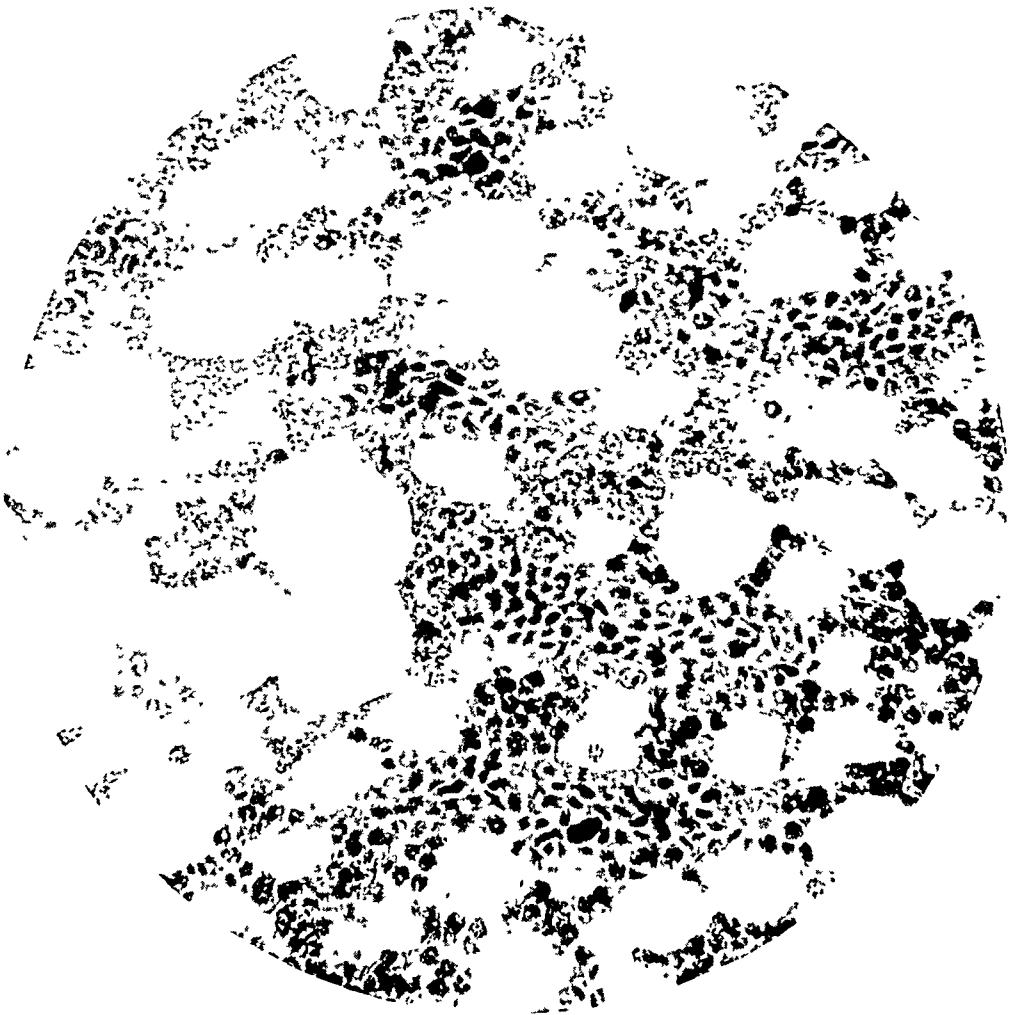


Fig. 10.—Appearance of marrow of a pigeon which had been given liver extract for eleven days, and which had been infected for seven days;  $\times 500$ . The reticulocytes on the day the bone marrow was taken were 16 per cent.

infection, then the superimposed infection, causing active stimulation of both red and white blood cells. The difference in composition of these foci of red blood cells is illustrated in figure 9, *B* and *C*.

Figure 10 shows the bone marrow of a bird that received liver extract for eleven days and was killed on the seventh day after infection. The reticulocytes in the peripheral blood were just beginning to increase

in number. The stimulation of red blood cells from the infection was prominent, although the erythrocytic foci did not seem to be as large as on the seventh day of infection in birds receiving no liver extract.

To determine whether liver extract influenced the extension of both red and white blood cells in the marrow to any marked extent when infection was superimposed, six birds were selected and matched as to weight and composition of the blood. To three of these animals liver extract was given for ten days; then all the animals were infected with the same culture of *Staph. aureus*. The pigeons were then put to death in pairs after twenty-four, forty-eight and seventy-two hours. In the birds receiving liver extract, no extension of the centers of production of red blood cells to the radial marrow was noted. The superimposed infection, however, had caused an early stimulation of both red and white blood cells. The reaction in all the birds seemed to be commensurable, as far as could be judged, with the intensity of the infection in each individual animal, regardless of whether the bird had received liver extract or not. The intensity of the infection was judged by the appearance of illness in the bird and the degree of anemia resulting from the infection.

#### COMMENT

In analyzing the results obtained it is essential to keep in mind that the bone marrow of the pigeon functions on a more immature level than that of many laboratory animals and man. This is reflected in the peripheral blood, where normally all the red blood cells are nucleated and the reticulocytes under the standard laboratory conditions mentioned average from 10 to 12 per cent. The bone marrow likewise contains a considerable number of megaloblasts, the youngest recognizable red blood cells. Also the radial bone marrow in the healthy adult pigeon is mainly fatty, and therefore hyperplasia of this marrow would indicate extension of the blood-forming tissue.

In acute pyogenic infection in pigeons, an increase in the number of leukocytes in the blood and an active stimulation of the white blood cells in the bone marrow were observed in most cases. A few exceptions occurred in which the animal died within forty-eight hours from an overwhelming infection. The reticulocytes of the peripheral blood in pigeons surviving the infection fell to a subnormal level at the height of the infection and later rose to levels above normal. The changes in the bone marrow corresponding to these alterations in the peripheral blood were an active new formation of immature erythrocytes, or megaloblasts, in the intersinusoidal capillaries, and extension of erythropoietic tissue into the radial bone marrow. The early stages of bone marrow growth corresponded to the decline of reticulocytes in the peripheral blood. The erythropoiesis then increased with great rapidity,

and bone marrows obtained after the seventh day of infection contained large islands of erythrocytes with a considerable number of mature cells. This phase of bone marrow activity was reflected in the peripheral blood by an increase in the percentage of reticulocytes, which were of greater immaturity than is customarily seen in the blood of normal pigeons. Consequently, infection caused a stimulation not only of white blood cells but also of red blood cells, the various stages of which could be followed with ease. This reaction of the red blood cells to an infection may be due to a circulating toxin acting directly on the inter-sinusoidal endothelium. It appeared, however, to be an indirect response to a diminution in the number of circulating red blood cells and in the hemoglobin content caused by increased destruction of blood. That an acute self-limited infection, such as pneumonia in man, may produce the same results is suggested from observations that I have made<sup>8</sup> on the reticulocytes in the peripheral blood.

In correlating the reticulocyte response and the condition of the bone marrow in pigeons after the daily administration of liver extract for some days, a surprising fact, suggested by former work,<sup>9</sup> was confirmed, namely, that the reticulocyte response is not accompanied by a stimulation and extension of the erythropoietic tissue. Rather there is a diminution of the quantity of the megaloblasts therein as well as of total erythropoietic tissue, and, as far as could be judged from counts on stained bone marrow sections, a diminution in mitotic figures. It is also of interest to note that the reticulocytes at the peak of their rise in the peripheral blood after the administration of the principle effective in pernicious anemia rarely exceed 20 per cent in contrast with from 25 to 35 per cent after an acute infection with *Staph. aureus* in healthy birds. It is evident that the mechanism of these reactions must differ fundamentally. The reticulocyte reaction to the infection is easily explained. Ample evidence of stimulation and new formation of erythropoietic tissue was present in the bone marrows. The mechanism of the reticulocyte response to the liver extract is, however, not so obvious. The logical conclusion seems to be that liver extract influences only a megaloblastic marrow and produces a reticulocyte response commensurable with the megaloblastic tissue present, but does not cause a stimulation or extension of erythropoietic tissue. The average percentage of the reticulocytes, 20 per cent, at the peak of their rise in pigeons after the administration of the principle effective in pernicious anemia may indicate that in the normal bird there is an average amount of megaloblasts, or immature cells, that undergo simul-

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8. Muller, G. L.: Unpublished observations.

9. Muller, G. L.: *Am. J. Physiol.* **82**:269, 1927.

taneous metamorphosis. A normoblastic marrow is thus produced in pigeons, a type of bone marrow that probably is abnormal for this animal.

The same reaction that has been observed in pigeons after the administration of the principle effective in pernicious anemia occurs also in man when megaloblastic hyperplasia is present, with regard to both the reticulocytes and the histologic character of the bone marrow. The reticulocyte response obtained in patients with pernicious anemia varies indirectly with the level of the red blood cells, which is apparently proportional to the megaloblastic tissue present. Peabody<sup>10</sup> admirably demonstrated that, in cases of pernicious anemia, the bone marrow, as a result of adequate liver therapy, returns toward normal. This consists in a qualitative change from a dominantly megaloblastic tissue to a dominantly normoblastic bone marrow, the functionally efficient type in adult man. There also occurs a quantitative decrease of the active bone marrow.

It has been suggested by Wright and Arthur<sup>11</sup> that this metamorphosis of the megaloblastic marrow removes a retarding influence on the multiplication of the primitive erythroblast, resulting in more frequent divisions of this particular type of cell. The experimental results in pigeons as well as the therapeutic results in man show a simultaneous metamorphosis of the megaloblasts (primitive erythroblasts), but this is accompanied by few or no signs of activity in the endothelium of the intersinusoidal capillaries of the pigeon's erythropoietic tissue. Nor does the microscopic picture of the bone marrow of patients with pernicious anemia in various stages of remission indicate that there is an increased activity in primitive cells. Rather the activity of the primitive erythropoietic tissue in pigeons is definitely decreased, at least temporarily.

With the conception of the two altogether different reactions produced in the bone marrow by infection and the principle effective in pernicious anemia, the inhibitory effect of infection on the action of the potent principle in pernicious anemia becomes explainable. It may be seen from the experiments recorded here, in which the influence of infection was superimposed on that of liver extract, that in practically every instance the reaction to the infection was the predominating feature, although, as was to be expected, the results were of two types. The interruption and prevention of a reticulocyte rise in response to liver extract by an acute infection were repeatedly demonstrated, with a subsequent larger reticulocyte response after the infection had subsided than could be ascribed to liver extract alone. This might be

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10. Peabody, F. W.: *Am. J. Path.* **3**:179, 1927.

11. Wright, G. P., and Arthur, B.: *J. Path. & Bact.* **33**:1017, 1930.

ascribed to an increase of the megaloblastic tissue caused by the infection. The identical interruption of a reticulocyte rise by acute cystitis in a patient with pernicious anemia and the subsequent rise to a higher level after the infection had subsided have been recently reported by Smithburn and Zerfas.<sup>12</sup>

It therefore seems probable that the inhibitory effect of infection on the action of the principle effective in pernicious anemia is due to the enormously increased activity of the primitive cells of the erythropoietic tissues resulting from infection, which liver extract in ordinary doses is unable to control. Apparently, acute infection produces temporarily a bone marrow of the megaloblastic type, the type that seemingly can be transformed into a normoblastic marrow in uncomplicated pernicious anemia and in normal pigeons by the addition of the principle effective in pernicious anemia. One may perhaps speak of the principle effective in pernicious anemia as a controlling substance that is either lacking or present in insufficient amounts in patients with pernicious anemia, and present in pigeons in small amounts proportional to other substances favoring a megaloblastic type of bone marrow.

#### SUMMARY

Experiments on pigeons dealing with the influence on the reticulocytes and the bone marrow of an acute infection, liver extract no. 343 (N. N. R.) and acute infection induced during the administration of liver extract have been described.

Intramuscular administration of broth cultures of *Staph. aureus* produced an initial fall of reticulocytes in the peripheral blood, followed by a rise above the normal level. The bone marrow showed active stimulation and extension of both red and white blood cells. The phase of decreased number of reticulocytes in the peripheral blood corresponded to rapid growth and division of immature red blood cells in the bone marrow.

The oral administration daily of liver extract for short periods also produced a reticulocyte response. The bone marrow, however, was quantitatively reduced and qualitatively changed from a marrow with many megaloblasts, or primitive young red blood cells, to a late erythroblastic and normoblastic marrow with a diminution of mitotic figures.

Infection superimposed after and during the feeding of liver extract caused a decrease in the number of reticulocytes in the peripheral blood comparable to that found in birds with infection only. Reticulocyte responses to liver extract were interrupted. The bone marrow showed a mixed picture in which the response to liver extract was distinguishable from that to infection, with the latter reaction predominating.

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12. Smithburn, K. C., and Zerfas, L. G.: *Ann. Int. Med.* 4:1108, 1931.

The underlying mechanism of the action on the bone marrow of substances effective in pernicious anemia was found to be diametrically opposite to the influence of infection. Liver extract transformed a megaloblastic bone marrow to a more mature level, while acute infection stimulated the primitive cells, or the endothelial cells of the inter-sinusoidal capillaries, to active new formation of megaloblasts. The neutralizing effect of infection on the action of substances effective in pernicious anemia thus seems explainable.

The reticulocyte response, the bone marrow changes and the influence of infection as observed in patients with pernicious anemia after the administration of potent material have been produced experimentally in pigeons, an animal peculiarly sensitive to substances effective in pernicious anemia.



# FIBROMYOMA OF THE BREAST

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In a recent discussion of benign tumors of the breast, Kleinschmidt<sup>1</sup> differentiates sharply between those of the nipple and skin and those of the breast proper. In the first group are hemangioma, fibroma, lipoma and rarely myoma. In the second group are adenoma, fibro-adenoma and fibroma, but not myoma.

Ten cases of leiomyoma of the nipple have been reported by Virchow,<sup>2</sup> Sokolow,<sup>3</sup> Niklas,<sup>4</sup> Riegel,<sup>5</sup> Hiebaum,<sup>6</sup> Bland-Sutton,<sup>7</sup> Bauer,<sup>8</sup> Kaufmann,<sup>9</sup> and Driak and Sternberg.<sup>10</sup> Reviews by Lindfors,<sup>11</sup> Lieber,<sup>12</sup> Schauder<sup>13</sup> and Driak and Sternberg<sup>10</sup> cover these cases. Bauer<sup>8</sup> studied the anatomy of the smooth muscle of the nipple that gives rise to these tumors.

Does myoma of the breast proper occur? Recent books on mammary tumors or on tumors in general do not mention it (Cheatle and Cutler,<sup>14</sup> Ewing<sup>15</sup>). A few cases have been reported, however, which deserve analysis. Klob<sup>16</sup> in 1864 mentioned two cases of what he called fibroma; an occasional muscle fiber was seen here and there. It is not clear whether in these cases the tumor was of the breast proper, since

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From the Department of Pathology, Cook County Hospital; Dr. Richard H. Jaffé, director.

1. Kleinschmidt, O.: *Chirurg* **3**:297, 1931.
2. Virchow, R.: *Virchows Arch. f. path. Anat.* **6**:525, 1854.
3. Sokolow: *Virchows Arch. f. path. Anat.* **58**:316, 1873.
4. Niklas: *Inaug. Diss., Würzburg*, 1889.
5. Riegel, A.: *Sborn. lék.*, 1891, vol. 4, pt. 3.
6. Hiebaum, A.: *Prag. med. Wchnschr.* **20**:314, 1895.
7. Bland-Sutton: *Practitioner* **59**:459, 1897.
8. Bauer, T.: *Beitr. z. path. Anat. u. z. allg. Path.* **62**:233, 1916.
9. Kaufmann, E.: *Pathology* (English translation), Philadelphia, P. Blakiston's Son & Co., 1929, p. 1782.
10. Driak, F., and Sternberg, H.: *Deutsche Ztschr. f. Chir.* **207**:352, 1927.
11. Lindfors, A. O.: *Monatschr. f. Geburtsh. u. Gynäk.* **11**:763, 1900.
12. Lieber, K.: *Beitr. z. path. Anat. u. z. allg. Path.* **60**:449, 1915.
13. Schauder, H.: *Deutsche Ztschr. f. Chir.* **205**:58, 1927.
14. Cheatle, G. L., and Cutler, M.: *Tumors of the Breast: Their Pathology, Symptoms, Diagnosis and Treatment*, Philadelphia, J. B. Lippincott Company, 1931.
15. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1931.
16. Klob, J. M.: *Pathologische Anatomie der weiblichen Sexualorganen*, Vienna, W. Braumüller, 1864, p. 492.

there is no description or report. Ribbert<sup>17</sup> mentioned a case, but also with no description or report. Ribbert also cited a case reported by Abramow.<sup>18</sup> This, however, proved to be an adenomyoma. Schauder<sup>13</sup> recently reported a case, but from the description and photomicrograph it is undoubtedly a sarcoma. Driak and Sternberg are essentially in accord with these conclusions. It appears that Kleinschmidt was apparently correct in excluding myoma from the benign tumors of the breast.

Unfortunately these authors did not have access to a case reported by Strong.<sup>19</sup> His case is an exception in that it is one of myoma of the breast proper, and in its clinical, gross and microscopic features resembles the following case in every detail.

#### REPORT OF A CASE

A 45 year old white woman entered the Cook County Hospital on March 2, 1932, with the complaint of a lump in the right breast. This lump had been present for fifteen years, had been painless, and was only very slowly getting larger. About two months before her admission to the hospital, the lump became painful and began to enlarge more rapidly. There had been no discharge or bleeding from the nipple. The patient had been pregnant several times, but had always aborted before one or two months. For the last eighteen months she had been having menstrual irregularity.

On physical examination, the lower half of the right breast was found to be occupied by a large, firm tumor mass the size of a small grapefruit. The tumor was independent of the nipple. The overlying skin was slightly discolored. There was no axillary or supraclavicular adenopathy; there were no other findings of significance.

The impression then was of a carcinoma of the breast. At operation, a simple mastectomy was done, including the breast, the nipple and the tumor mass, and the specimen was sent to the laboratory for a rapid frozen section. This revealed a benign fibromyoma, and no further resection was done. The patient made an uneventful recovery and went home on the seventeenth postoperative day.

The breast tissue was almost entirely replaced by the tumor, which was very firm but slightly elastic, well encapsulated, and entirely distinct from the nipple, the margin being 2 cm. from the nipple (fig. 1). The cut surface was grayish white and trabeculated. Near the center was an irregular cystic area, 2 by 2 by 3 cm. in dimensions, filled with blood-tinted fluid. The tissue at the margin of this cystic area was edematous and mottled with deep red areas.

Frozen and paraffin sections from nine different areas of the tumor stained by hemalum-eosin, van Gieson's stain and Mallory's phosphotungstic acid hematoxylin method revealed the tumor to be composed of smooth muscle cells arranged in interweaving bundles separated by a moderate amount of connective tissue (fig. 2 A). There was a tendency toward perivascular foci of proliferation. In some areas this was pronounced, the muscular wall of the blood vessels blending indistinguishably into the surrounding concentrically arranged tumor tissue (fig. 2 B). The cells stained yellow by van Gieson's stain and purple-blue by the

17. Ribbert, M. W. H.: *Geschwülstlehre*, Bonn, F. Cohen, 1914, vol. 9, p. 427.

18. Abramow, S. S.: *Zentralbl. f. allg. Path. u. path. Anat.* **12**:926, 1901.

19. Strong, L. W.: *Am. J. Obst. & Gynec.* **68**:53, 1913.

Mallory method, the connective tissue stained red by both methods. The nuclei were fusiform, with rounded ends, distinct nuclear membrane, and fine evenly distributed chromatin granules. There was no anaplasia. Very few mitotic figures were seen. The Mallory method brought out the fine intracellular myofibrils characteristic of mature smooth muscle cells. No glandular elements could be found. In the region of the cystic area various degenerative changes could be followed. Thrombosis of numerous blood vessels was seen. This was followed by atrophy and necrosis of many of the muscle fibers and small areas of hemorrhage. There was then an overgrowth of connective tissue which became

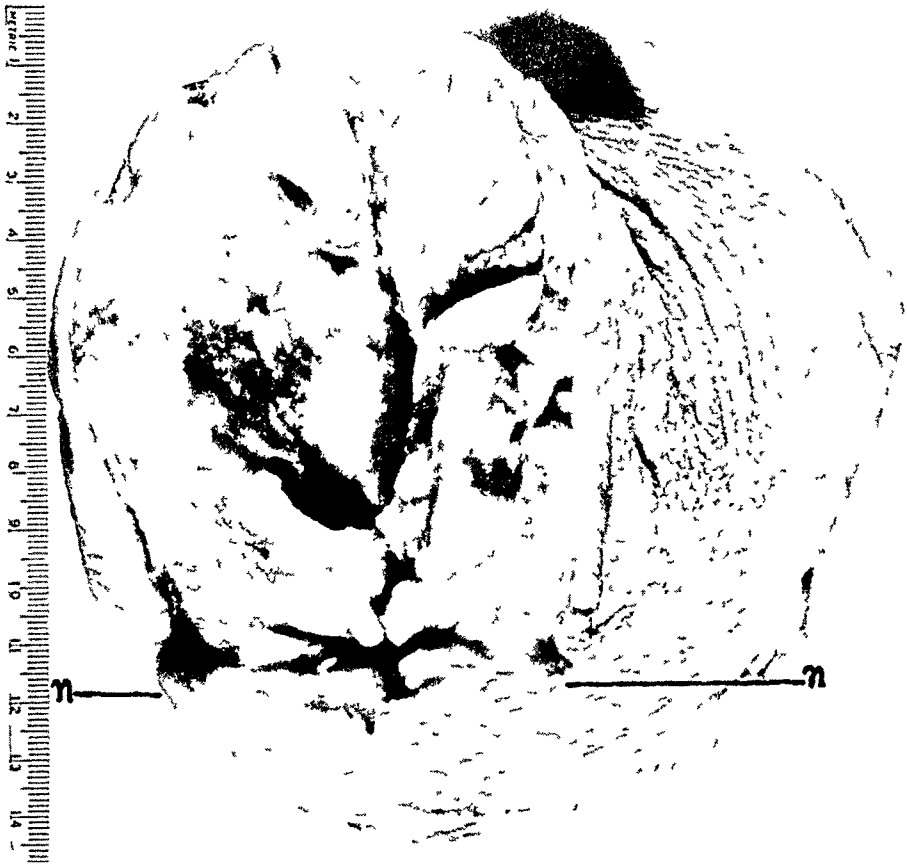


Fig 1—Fibromyoma of the breast. The nipple (*n*) has been cut through; its separation from the tumor is distinct.

edematous and loosened. Small cystic areas developed, which finally fused together to form the central cystic cavity. Diagnosis: Fibromyoma of the breast with cystic degeneration.

#### COMMENT

The histogenesis of this tumor is of interest. There are four possibilities.

It may be teratoid in origin, with extreme overgrowth of the myomatous element. The pseudomucinous cystadenoma of the ovary and the struma ovarii are such tumors; occasionally in teratoma of the testis

and in mixed tumors of the salivary glands one element predominates. Chondroma and similar tumors of the breast are considered to be of teratoid origin, and this tumor may be of similar nature.

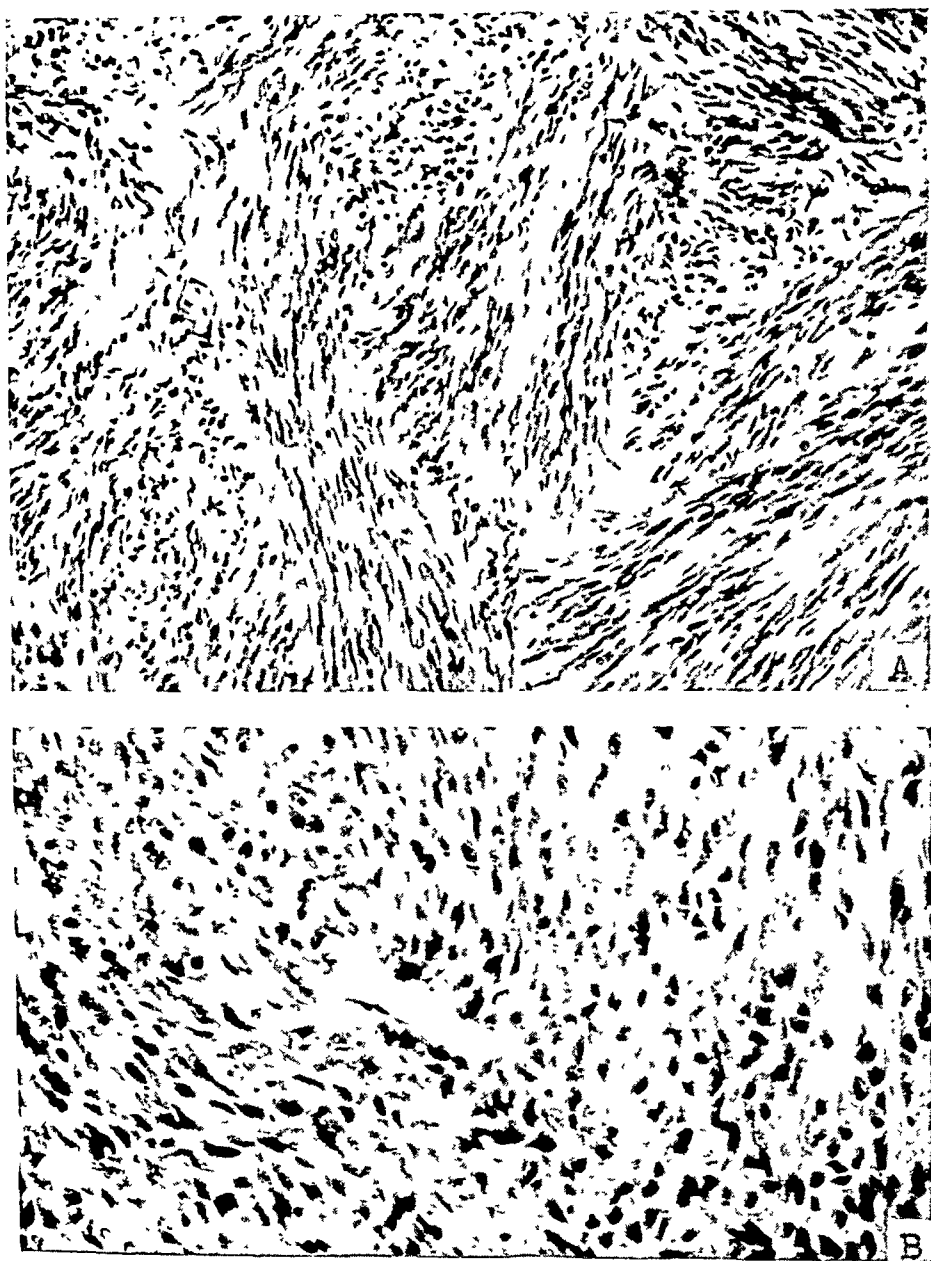


Fig. 2.—Fibromyoma of the breast. *A*, interweaving bundles of smooth muscle cells; no anaplasia and no mitotic figures; Hemalum-eosin. *B*, fusion of the muscular wall of the blood vessel with the surrounding tumor tissue; van Gieson's stain.

A second possibility would be embryologically displaced smooth muscle from the nipple. Fibro-adenoma of the breast has been con-

sidered to be the result of embryologic defects; a similar explanation may apply here.

A third theoretical possibility is the "myo-epithelial layer" of the French school of Peyron, Corsy and Surmont.<sup>20</sup> The sweat glands have an incomplete layer of smooth muscle applied against the epithelium, which seems to regulate secretion. From embryologic data these workers correlate this muscle layer with the outermost of the two layers of epithelium lining the ducts of the breast. They are able to recognize tumors arising from this layer, which are frequent in animals but occur occasionally in the human being. This conception is at the present time still in a theoretical stage.

A fourth possibility appears to be the most logical: an origin from the muscular layer of the blood vessels. The perivascular foci of proliferation and the fusion of the muscular layer of the blood vessels with the tumor tissue speak in favor of this. Strong's case had a marked similarity in this respect.

#### SUMMARY

A case of fibromyoma of the breast is reported. This is a true fibromyoma, having no glandular elements, and is of the breast proper, having no relation to the nipple. One similar case has previously been reported. The histogenesis offers four possibilities: teratoid origin with overgrowth of the myomatous element; embryologic displacement of smooth muscle from the nipple; an origin from the "myo-epithelial layer" of the French school, and that from the muscular layer of the blood vessels. The last is apparently the true source of the tumor.

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# TUBEROUS SCLEROSIS

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AND

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PHILADELPHIA

Tuberous sclerosis is a rare congenital, sometimes hereditary and familial disease of unknown etiology, characterized by mental deficiency, epilepsy, tumors of various tissues, developmental defects and a histologically distinctive lesion of the brain which may involve any lobe, any surface or any folium and is often associated with glial masses that hang in the ventricles like "candle gutterings." It occurs uncommonly in the United States, more frequently in Australia<sup>1</sup> and Great Britain, and is confined largely to Europeans,<sup>2</sup> especially those of the poorer classes.<sup>3</sup> Syphilis,<sup>4</sup> tuberculosis and neuropathic tendencies<sup>5</sup> have been noted in the parents in a number of cases. The persons with this disease almost always show mental impairment, which occasionally may be so slight that it goes unrecognized. Psychically, they may be normal<sup>6</sup> and later regress, but are usually imbeciles or idiots from birth. Epilepsy generally begins in infancy, although it may not appear for a number of years, when it is apt to precipitate the onset of the final phase. The attacks increase in frequency and duration in the later stages, varying considerably in their clinical characteristics,<sup>7</sup> simulating at times idiopathic<sup>8</sup> or jacksonian<sup>9</sup> epilepsy. Death may occur in status epilepticus, or postepileptic mania may follow.<sup>2</sup> Other nervous symptoms are rare.<sup>10</sup> Delay in talking, walking and dentition are the rule, and various stigmas of degeneration have been recognized, of which the high narrow palate is perhaps the most common. Optic atrophy, cata-

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From the Pathological Department of the Jefferson Medical College and Hospital, the Pathological Department of the Philadelphia General Hospital and the Pediatric Department of the Jefferson Medical College and Hospital.

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ract and retinal tumor (the so-called phakoma) are occasionally the only clinical manifestations.<sup>11</sup> The cerebral and cerebellar lesions consist of pearly white, gray or yellowish-gray, hard nodules; these may be no larger than a pea, or they may involve half of a hemisphere. They are well defined, principally confined to the surface, and they cause atrophy of adjacent gyri. Associated with them are concretions, cystic cavities, glial or vascular tumors and meningitis. Microscopically, the altered glial cells and astrocytes make a dense fibrillar network, which surrounds peculiar giant cells, interrupts the cyto-architecture of the cortical layers and imparts a notable density to the hypertrophic cortical nodule. There are agenesis of myelin sheaths and persistence of the external granular layer of the cerebral cortex. Cutaneous lesions arise about the nasolabial folds or elsewhere on the face, although seldom below the clavicles;<sup>12</sup> they consist of fibroma-like nodules of the Hallopeau-Leredde type, simple adenomas of sebaceous glands<sup>13</sup> or altered hair follicles.<sup>14</sup> The tumors associated with this condition, found in the brain, ependyma, kidneys, heart, skin, pancreas, intestine, thyroid gland and mammae, may be undifferentiated embryonic neoplasms, gliomas, hemangiomas, lipomas, myomas, fibromas, mixed tumors, teratomas, dermoid cysts or carcinosarcomatodes. The disease is uniformly fatal. Without an autopsy it is often unrecognized or incorrectly diagnosed. Early descriptions were made by von Recklinghausen,<sup>15</sup> Virchow<sup>16</sup> and Bourneville.<sup>17</sup> Subsequently Sherlock<sup>18</sup> proposed the name epiloia, which has been taken up and used extensively by the British. Important contributions have been made by Bundschup,<sup>19</sup> Freeman,<sup>20</sup> Bielschowsky,<sup>21</sup> Babonneix,<sup>22</sup> Bolsi,<sup>23</sup> Kreyenberg, Delbanco and Haack,<sup>24</sup> Lazar,<sup>25</sup> Globus<sup>26</sup> and Turville.<sup>27</sup>

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## REPORT OF CASES

CASE 1.—R. C., a 4 months old Negro girl, weighing 8 pounds (3,600 Gm.), was admitted to the Philadelphia General Hospital on March 31, 1927, in the service of Dr. C. A. Fife. The father and mother were living and well. The mother had had three pregnancies, one of which terminated in abortion, and the other two in normal deliveries. The first child, a girl, died of pneumonia at the age of 1 year. The second failed to gain weight, but was otherwise apparently healthy until the night before admission, when she was seized with a severe convulsion, followed by rigidity, tremor and fever. On examination, she presented the physical signs of bronchopneumonia, craniotabes, bulging of the fontanel, twitching of the eyes and spasticity of the muscles. The pupils were equal and in midsyllation, but did not react to light or to accommodation. For two months, convulsions recurred frequently. The child failed to gain weight, was fretful, sensitive, refused to nurse, vomited frequently and perspired copiously, and her breathing was accompanied by a peculiar snorting sound. For a brief interval in June, 1927, some improvement was noted; nursing was resumed, convulsive seizures abated, and muscular rigidity largely disappeared. On relapse, all the previous symptoms returned with increased severity. Suffering from inanition, the patient contracted pneumonia and died on Jan. 24, 1928.

The afternoon temperature ranged between 101 and 102 F., rising to 105 F. with each attack of pneumonia; the pulse rate was from 120 to 160; the respirations, from 30 to 50.

The spinal fluid showed a pressure of 12 millimeters of mercury, 2 cells per cubic millimeter, a faint trace of globulin, a negative Wassermann reaction and a colloidal gold curve of 0.0111000000. The blood sugar was 0.80 Gm.; the blood urea, 37 Gm. The white blood cells numbered 12,600, polymorphonuclear leukocytes 57, lymphocytes 40 and monocytes 3. The urine was normal.

The clinical diagnosis was: congenital syphilis, tetany, bronchopneumonia and possibly syphilitic or tuberculous meningitis.

The therapy included administration of mercury, iodides and sulpharsphenamine, spinal drainages and exposures to ultraviolet rays.

Postmortem examination (Dr. R. B. Richardson) showed extreme emaciation; marked plantar flexion of the fingers and toes; exfoliation, scarring and depigmentation of the skin along the right nasolabial fold; along the left, numerous small, flat, closely set nodules; eruption of two normal upper and lower incisors; a normal condition of the scalp and meninges, and no enlargement of the brain. On palpation of the brain, nodules were located that projected slightly beyond the surface. They were sharply demarcated, with slightly roughened surfaces, and were of a rubber-like consistency. They occupied symmetrical positions in the mesial borders of the Rolandic areas and the posterior portions of the frontal lobes, extending over the convex and down into the mesial surfaces. The dimensions of the nodule in the right cerebral hemisphere were 3 by 5 cm., and that in the left, 2.5 by 4 cm. The convex surface and to a less extent the bases of the affected convolutions were broadened (fig. 1A). When incised, the nodules did not settle away from the knife as normal cerebral tissue does. The cortex was pale, mottled and imperfectly defined. The uninvolved gyri adjacent to the sclerotic ones were pink and atrophic, with shallow fissures. All the cerebellar folia were widened, and on palpation their consistency was identical with that of the cerebral nodules. The pons, medulla, pituitary gland, basal vessels and ventricles appeared normal. There was bilateral bronchopneumonia; the left suprarenal gland was twice as large as the right; all the mesenteric lymph nodes were enlarged; the thymus was small; the heart, aorta, spleen, liver and kidneys were normal.



CASE 2.—S. O., a white girl, was born in December, 1929. The father, mother and two older brothers were living and well. The mother's gestations and accouchements were normal. The day after birth the infant twitched continuously and appeared to be in a "trance." Convulsions lasting from three seconds to one hour began on the fifth day and recurred at frequent intervals. Following these, she would lie on the right side and alternately sleep forty-eight hours and remain awake fifteen hours. Her appetite, manner of eating and gain in weight were normal. She was taken to a hospital on Feb. 25, 1930. Her family physician stated that the convulsions were on the left side, and that on lumbar puncture bloody fluid had been withdrawn. While she was in the hospital, all the convulsions were on the right side. The right side of the face was involved, the head and eyes were drawn to the right, and it seemed that the right extremities, particularly the arm, were involved, and that both the right arm and leg were more spastic. Because of these discrepancies the child was closely observed for five days before active measures were taken. On repeated examinations the findings remained constant, and a decompression on the left side seemed indicated. This revealed no changes other than an overdilated subarachnoid space, from which a rather abnormal amount of fluid was obtained. The recovery was uncomplicated, and following the operation convulsions were less frequent. They were still on the right side. The child was discharged on March 19, 1930, with the suggestion that, after complete recovery from the effects of the operation, she should be returned for a right subtemporal decompression. It was noted (March 29) that the circumference of the head was 1 inch (2.5 cm.) greater than it had been prior to operation. Since developing hydrocephalus was suspected, it was thought wise not to operate. Two months later she was admitted to Jefferson Hospital. Her head was uniformly enlarged, the eyes moved continuously and independently of each other, and when disturbed the muscles became rigid. Vomiting occurred occasionally, but food was usually retained. Generalized rigidity was frequent and alternated with marked flaccidity and listlessness. On occasion, a typical hydrocephalic cry was heard. The chest, abdomen and reflexes were apparently normal, and the lymph nodes were not palpable. On June 29, 1930, signs of infection developed with fever (107 F.) and Cheyne-Stokes respirations. Death occurred two weeks later.

On roentgenographic examination of the brain, the soft parts appeared retracted near the anterior fontanel on the left side and in the region of the vault and occiput. Ventriculographic examination showed dilatation of the lateral ventricles but a perfectly free communication with the other ventricles. Roentgenographic examination of the spine and skull following injection of iodized poppy seed oil 40 per cent showed the ventricular system enlarged, although the third and fourth ventricles were not well isolated.

The hemoglobin content was 65 per cent; the red blood cells numbered 3,780,000; the white blood cells, 5,400. The color index was 0.98. The polymorphonuclears were 21; the small mononuclears, 72; the large mononuclears, 7. The spinal fluid was clear and contained 1 cell per cubic millimeter. The globulin was not increased; sugar was 32 mg. The Wassermann reaction was negative.

At postmortem examination, except for well marked lividity and rigidity, the trunk, limbs, skin, hair and genital organs were normal; the cranial incision appeared well healed. The skull was uniformly and proportionately enlarged. The right anterior half of the skull cap showed craniotabes, and adherent to it was a thickened, gray, tough, opaque dura. Considerable fluid had accumulated beneath the unadherent and unthickened pia. The brain weighed 790 Gm., and the entire anterior half of the right cerebral hemisphere was uniformly pale and hard. The

transition from pink uninvolved cerebral tissue to the sclerotic area was abruptly marked by extreme anemia and a cartilaginous consistency. These hard, anemic convolutions were broad, superficial and in some places umbilicated. They cut with increased resistance and on section presented a sharp edge and a pale, broad, dull, somewhat mottled, porous, poorly marked surface. The adjacent gyri were deformed and partially atrophied. The lateral ventricles were markedly dilated, chiefly in their posterior horns. There were no ventricular obstructions or tumors. The ependyma, pons, medulla, cerebellum, pituitary gland, basal vessels and third and fourth ventricles appeared to be normal. Permission to examine the contents of the chest and abdomen was not obtained.

MICROSCOPIC OBSERVATIONS IN CASES 1 AND 2.—Cerebral and cerebellar tissues in each case were fixed in alcohol, formaldehyde, Zenker's fluid and Müller's fluid. Some were frozen and sectioned; others were blocked in paraffin and celloidin, cut, and stained with phosphotungstic acid, Nissl's toluidine blue, scarlet red, hematoxylin and eosin, Weigert's stain for myelin, Bielschowsky's silver stain, Cajal's silver stain for neuroglia, Gram's stain for bacteria in tissues and Levaditi's stain for spirochetes.

The histologic features in the two brains were identical.

With Weigert's stain, myelinization was, of course, nowhere so abundant as in an adult brain. In the sclerotic patches it was practically absent (fig. 1 *A*). The silver preparations demonstrated that this was not due to absence of nerve fibers. There was loss of architecture, marked gliosis of the marginal layers and a lack of the sharp line of cleavage from underlying cortical layers (fig. 1 *B*). Neurofibrils were greatly increased and tended to form in clumps. Irregular and abnormal ganglion cells were present in the upper layers of the cortex and in the overlying marginal layers.

Sections prepared by Cajal's silver stain for neuroglia (fig. 1 *C* and *D*) showed enormous overgrowth of neuroglia, which formed a matted, interlacing network of fibers that extended through the granular layer upward into the subarachnoid space. Grouped directly beneath the space were collections of ganglion cells.

Along with the loss of architecture in the toluidine blue (Nissl) preparations (fig. 2 *A*) there was a resemblance to the deeper layers of the cortex in dementia paralytica. Neuroglia was increased, the number of ganglion cells was reduced, rod cells were present in great numbers, and small blood vessels were unduly prominent. In size and shape, the degenerated and necrotic ganglion cells varied considerably and, with their atypical dendrons, pointed in every direction.

In the sections stained with scarlet red, enormous quantities of lipoid were demonstrated in practically every cell. Phagocytes loaded with fat droplets were grouped about blood and lymph vessels, and many monster cells were seen loaded with lipoid material. In figure 2 *B*, one of these can be seen at the left of the center, and others, in the upper left hand corner. Neurofibrils from the granular layer extended into the subarachnoid space.

In the Bielschowsky preparations, the cytologic and morphologic variations were well brought out. In figure 2 *C*, a "large cell" with its processes is located just below the marginal layer and resembles the Betz cell of the motor cortex. An abundance of neurofibrils and atypical ganglion cells occupied the marginal layer, the boundaries of which were indistinct. The increased number and length of the cell processes made it difficult to demonstrate with any frequency or degree of accuracy the cytologic and fibrillar relationship. The differences in staining of the nuclei, protoplasm and processes of the "large cells" whereby neuroglial elements can be distinguished from ganglion cells were noted in a high percentage. Bolsi claimed to have demonstrated this much better with a modification of the silver nitrate pyridine method of Cajal.

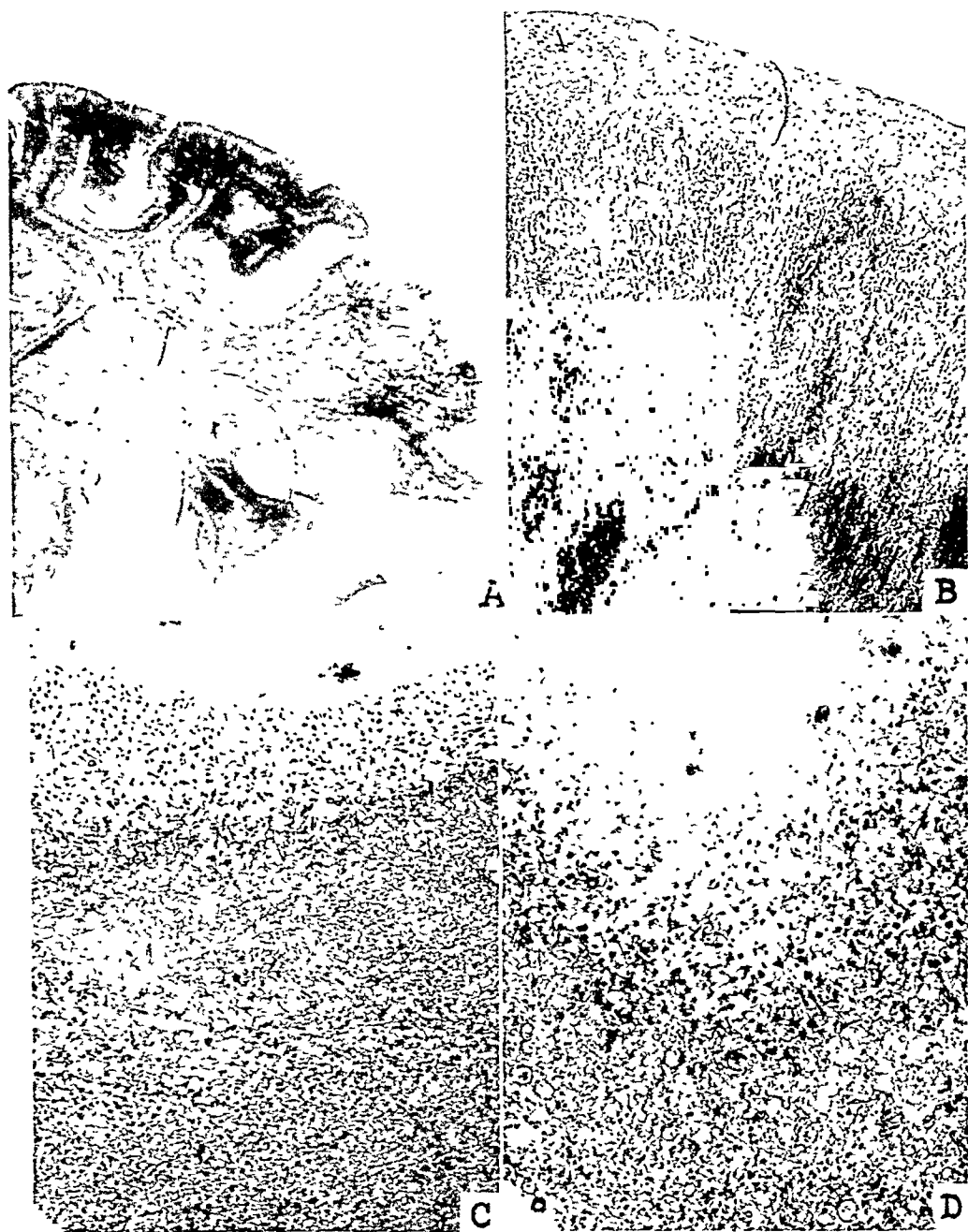


Fig. 1.—*A*, section showing hypertrophic and uninvolved gyri. The mottled appearance of the sclerotic patch on the right is characteristic of the lesion. It shades off into the normal convolutions on the left. Myelin sheath stain. *B*, section through a sclerotic patch, showing agenesia of myelin sheaths, loss of architecture and marked gliosis. Myelin sheath stain. *C*, section through a sclerotic patch, showing intense laminar gliosis. Silver stain for neuroglia (Cajal). *D*, same as *C* under higher magnification.

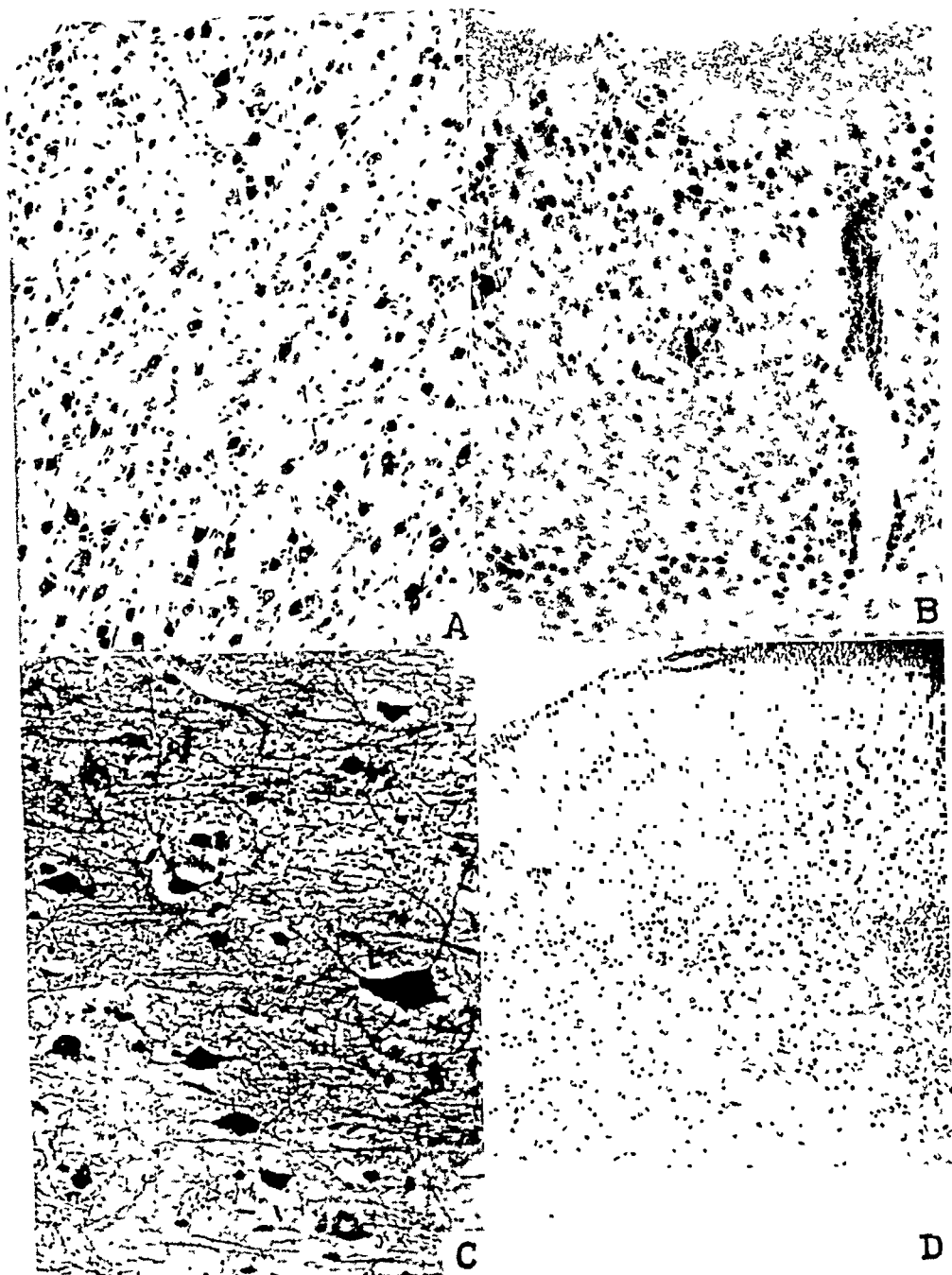


Fig. 2—*A*, section through a cortical sclerotic patch just below the marginal layer. Note the resemblance to the deeper cortical layers in a person with dementia paralytica. Toluidine blue (Nissl stain). *B*, section through a hypertrophic convolution. Note the enormous quantities of lipid, droplets of which can be seen in practically every cell. They can be better seen by magnifying the photograph with a hand lens. Fat-containing phagocytic cells are concentrated about a blood vessel to the right of the center. Scarlet red stain. *C*, cortex of hypertrophic convolution, showing part of marginal layer and upper cortical layers. Bielschowsky preparation. *D*, cerebellum, case 1. Note the gliosis of the molecular layer and the paucity of nerve cells in the granular layer. Except for the two wrinkled and distorted cells, the Purkinje cells are absent. Phosphotungstic acid stain.

The cerebellar sections in case 1 consisted entirely of areas of tuberous sclerosis, characterized by widened folia, concretions, sometimes partially or entirely calcified arteries, capillaries with proliferated endothelium and an atrophic molecular layer of varying thickness (fig. 2D). The latter was the seat of an intense, irregular gliosis, which formed a brushlike arrangement on the surface. Just proximal to this, a band of parallel fibers frequently began and stopped abruptly. No normal Purkinje cells were found. In their places were a few large, irregular, oval or polyhedral, more or less indistinct cells, which had shrunk away from the spaces they appeared previously to have occupied. Their nuclei were either absent, irregular, pyknotic or fragmented with increased chromatin granulation. Most of these cells were devoid of processes, but a few short, coarse, rudimentary ones were found. The cytoplasm stained poorly and contained irregular granules and small vacuoles. Identical cells were found in ectopic positions in the granular and medullary layers. The small round, deeply staining cells of which the granular layer is normally largely composed were found only sparsely in the affected areas. In their stead was a dense infiltration by cells indistinguishable from embryonic glial elements. They were scattered sparsely throughout the molecular and granular layers and were usually arranged perpendicular to the surface.

#### COMMENT

A family history of syphilis, tuberculosis, degenerative or neuropathic tendencies was not elicited in either case. The parents were below the age of 30 and seemed normal. The Wassermann reactions in both cases were negative, and none of the lesions characteristic of congenital syphilis or of tuberculosis was found. Since adenoma sebaceum is said to be much rarer in races other than the Caucasian, it is interesting that it was present in a Negro infant (case 1). The sebaceous glands, already present in the fetus, begin to secrete about the fourth year,<sup>28</sup> and adenoma sebaceum usually does not appear earlier, although it has been noted at 9 weeks,<sup>29</sup> 6 months<sup>3</sup> and in our case at 13 months. Except for the adenomas of the sebaceous glands, no tumors (limited autopsy, case 2), no stigmas of degeneration and no skin lesions such as fibroma molluscum, pedunculated polypi, pigmented nevi, "café au lait" spots or anemic or valvular nevi, which have been described by Yakovlev and Guthrie,<sup>30</sup> were noted. Both infants were of the third gestation, and both were girls. The gestations and accouchements were normal. There was no evidence of tuberous sclerosis in the brothers or sisters, although in case 1 only a guarded statement can be made. The course in both was characterized by a steadily progressive mental and physical deterioration, expedited by inanition and terminated by infection. There was no evidence of retinal tumors, of contractures or of visual or auditory impairment. Interest-

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ing ocular signs and craniotabes were noted in both cases. Copious perspiration, vomiting and interference with feeding were noted in case 1. Both infants suffered from epilepsy, and in one (case 2) the localizing signs were sufficient to justify surgical intervention. The shifting of the signs, which at first was attributed to discrepant statements, may be explained thus: The lesion was in the right hemisphere, and the original signs pointed to that area; with the advent of hydrocephalus and compression of the opposite side, the localizing signs shifted to the other side. The same phenomenon is often observed in cases of tumor of the brain.

As has been observed by others,<sup>31</sup> surgical intervention appeared not to influence the subsequent course.

As death occurred while both patients were still infants, the histologic changes in the lesions were not far advanced as judged by the absence of tumors and by the absence (case 2) and paucity (case 1) of concretions. In case 1, the calcific deposits were confined to the cerebellum, which coincides with the observation that when widespread they are apt to be more plentiful in that region. It was there, also, that two prominent characteristics of tuberous sclerosis were represented, viz., the glioblastomatous and the neurocytodysgenetic.<sup>21</sup> Enlargement of the brain, hydrocephalus and dural thickening were noted in case 2. The hydrocephalus was chiefly confined to the lateral ventricles and could not be accounted for on an obstructive basis, since the ventricular communication was intact. In some cases it has been ascribed to tumors that may be widespread although occurring commonly in the ventricles, the caudate nucleus and the stria terminalis. It is interesting in this connection that Freeman<sup>20</sup> was not convinced that any causal relationship existed in his case between the ventricular hydrocephalus and a tumor that lay adjacent to the foramen of Monro. Cysts have been found in the occipital lobe away from the sclerotic lesions or occasionally in the frontal lobe in the midst of a tuberous area. They were not definite in either of our cases, although in case 2 the porous nature of the cortex suggested them. In case 2, the entire anterior half of the right cerebral hemisphere was converted into an area of tuberous sclerosis, and the altered histologic picture extended well down into the fissures, which is uncommon.<sup>32</sup> In case 1, the cerebral nodules were small, but the rare cerebellar lesion was so extensive that definitely normal structure was not encountered. In case 1, the left suprarenal gland was twice as large as the right. It is unfortunate that the restrictions on the autopsy in case 2 prevent a more positive statement regarding visceral tumors. Clinically, there was no evidence of them, but, as Hyman<sup>33</sup> has shown, they may exist unrecognized.

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## ETIOLOGY

Bourneville,<sup>17</sup> Fürstner and Stuhlinger<sup>34</sup> and Brushfield and Wyatt<sup>2</sup> believed tuberous sclerosis to be an inflammatory lesion. In some cases, the parents have been tuberculous or syphilitic,<sup>4</sup> and in a few cases the Wassermann reaction of the blood has been positive. Campbell<sup>35</sup> considered it an evolutionary disturbance, beginning not before the seventh fetal month, which is the time at which the plan of the sulci and gyri is laid down. Vogt,<sup>36</sup> Globus,<sup>26</sup> Bielschowsky<sup>21</sup> and Hartdegen<sup>37</sup> regarded it as a malformation, a blastoma or an inseparable combination of the two, and attributed the inflammatory changes to alterations taking place in the areas of tuberous sclerosis. Bielschowsky especially emphasized these combined manifestations and stressed the probable interrelationship of syringomyelia, solitary gliomas and von Recklinghausen's disease (peripheral spongioblastosis) with tuberous sclerosis (central spongioblastosis). Yakovlev and Guthrie pointed out further clinical analogies with von Recklinghausen's disease, cerebral angiomas and vascular nevi of the skin, suggesting that these were ectoblastic malformations to be classified under the term "congenital ectodermoses" along with the congenital ectodermal dystrophies<sup>38</sup> and dysplasias.<sup>29</sup>

A striking feature of tuberous sclerosis is manifested in the lipoforous tissue disturbances. Scattered through the sclerosed nodules and grouped about blood and lymphatic vessels are large phagocytic and monster cells loaded with lipid droplets. In every case there is agenesis of myelin sheaths, and in many there are adenomas of sebaceous glands, renal and mammary lipomas, dermoid cysts and lesions of the suprarenal glands consisting of atrophy and aplasia of the cortical cells, fibrosis of the medulla or, as in case 1, hypertrophy of one and atrophy of the other gland. These changes suggest an embryologic metabolic imbalance, and this opinion is further supported by the work of Steinbiss<sup>39</sup> in his studies of rhabdomyoma of the myocardium. Theorizing along this line seems inappropriate at this time. Pathologic data assembled so far in this condition point so strongly to a metabolic imbalance that metabolic studies and administration of the suprarenal cortical hormone,<sup>40</sup> as a trial, seem warranted.

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## SUMMARY

The clinical and pathologic features of two cases of tuberous sclerosis are presented and discussed. One of the cases, which was complicated by adenoma sebaceum, occurred in a Negro infant.

In obscure cases of obstructive or nonobstructive hydrocephalus associated with epilepsy with or without adenoma sebaceum, tuberous sclerosis must be considered as a possibility.

The features of lipodystrophy are emphasized, and it is suggested that tuberous sclerosis is a congenital metabolic disturbance.



# BLOOD CYSTS ON THE HEART VALVES OF NEW-BORN INFANTS

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AND

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Small, circumscribed, elevated nodules on the heart valves of new-born infants were reported as early as 1844 by Elsässer<sup>1</sup> and 1857 by Luschka.<sup>2</sup> Since then many similar observations have been recorded, but very few in other than the German or the French medical literature. There has been considerable controversy as to the nature of these nodules, first one view predominating, then another as later studies superseded earlier ones.

## INCIDENCE

Elsässer<sup>1</sup> found the nodules in more than half of infants examined between birth and the twenty-third day of life. Luschka<sup>2</sup> reported a 25 per cent incidence. Parrot<sup>3</sup> considered the nodules as an almost invariable finding, reporting their occurrence in 103 of 120 infants examined post mortem in the first few months of life. In Fahr's<sup>4</sup> series of infants in the first few months of life, the nodules occurred almost constantly; in those in the second half of the first year, the incidence diminished to 50 per cent, and in those past the first year, the nodules occurred rarely. Haushalter and Thiry,<sup>5</sup> Wegelin<sup>6</sup> and Jonsson<sup>7</sup> cited an incidence of, respectively, 78 per cent, 66 per cent and 63 per cent. While the greatest frequency of occurrence is in the very first few months of infancy, a number of cases occurring in childhood and adult life have been reported—namely, by Haushalter and Thiry,<sup>5</sup> Bundschuh<sup>8</sup> and Wegelin.<sup>6</sup>

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1. Elsässer, cited by Luschka.<sup>2</sup>
2. Luschka, H.: *Virchows Arch. f. path. Anat.* **4**:171, 1852; **11**:144, 1857.
3. Parrot, M. G.: *Arch. de physiol. norm. et path.* **1**:538, 1874.
4. Fahr, E.: *Virchows Arch. f. path. Anat.* **184**:274, 1906.
5. Haushalter, P., and Thiry, C.: *Arch. de méd. expér. et d'anat. path.* **10**: 558, 1898.
6. Wegelin, C.: *Frankfurt. Ztschr. f. Path.* **2**:411, 1908; **9**:97, 1912.
7. Jonsson, S.: *Virchows Arch. f. path. Anat.* **222**:345, 1916.
8. Bundschuh, E.: *Frankfurt. Ztschr. f. Path.* **6**:65, 1911.

The valves of predilection for the distribution of the blood cysts are the mitral and the tricuspid, on which the nodules occur with about equal frequency. They are found less often on the pulmonic, and infrequently on the aortic, leaflets. Wegelin<sup>6</sup> recorded the following distribution: On the mitral valve alone the nodules were found ten times; on the tricuspid valve alone, six times; on the mitral and the tricuspid valves, thirteen times, and on the tricuspid and the pulmonic valves, one time.

Jonsson<sup>7</sup> described nodules in forty-five of seventy-one cases in which postmortem examination was performed. The distribution of nodules on the various valves is recorded in the table.

In sixteen consecutive postmortem examinations of infants (the premature as well as the full-term, and the stillborn as well as those born

*Distribution of Nodules on the Heart Valves in Jonsson's Series of New-Born Infants*

	Valves Showing Nodules	Number of Cases
Mitral, tricuspid, pulmonic and aortic	4	2
Mitral, tricuspid and pulmonic	3	2
Mitral and tricuspid	2	23
Tricuspid and pulmonic	2	1
Tricuspid and aortic	2	1
Mitral and aortic	2	1
Mitral	1	7
Tricuspid	1	6
Aortic	1	1
Pulmonic	1	1
Total		45

living) twelve showed blood cysts on the valves. A similar high incidence is recorded in the various published reports.

We have seen, on the leaflets of the heart valves of new-born infants, small, circumscribed, elevated, dark red nodules. These elevations appear grossly to be small cysts filled with blood. When present, they are almost invariably seen on both the mitral and the tricuspid leaflets, infrequently on the pulmonic leaflets and rarely on the aortic. The nodules occur as elevated, circumscribed, spheroid globules, presenting a dark red color, and varying from pinpoint to pinhead in size, seldom exceeding 1 mm. in diameter (fig. 1). They vary in number from two or three to ten or fifteen, though as many as thirty have been reported. The nodules project above the auricular surface of the auriculoventricular leaflets near the free margin, between the edge and the line of contact on closure. On the semilunar leaflets, the nodules project into the ventricle and are located at or very close to the line of attachment of the leaflet.

## HISTOLOGIC DISCUSSION

On histologic examination, the nodules as seen in cross-section appear as monolocular or bilocular or even multilocular spaces filled with red blood cells (fig. 2). The partitions dividing the spaces into several chambers are not constantly complete, thus there is obvious communication between compartments. The spaces are lined by a single layer of endothelial cells, in appearance similar to the surface endothelium of the valve leaflets. The cells are flattened and show well staining,



Fig. 1.—A heart showing blood cysts on the mitral valve, on the auricular surface near the junction of the chordae tendineae with the leaflet;  $\times 2$ .

elongated nuclei. The spaces present the appearance of cystlike enlargements protruding above the surface level of the leaflet. The projecting surface of the nodule and the corresponding side of the leaflet present a smooth, uniformly even outline except for the elevation of the nodule above the surface level, while the opposite side is irregular and uneven, showing numerous indentations of the surface endothelium into the stroma of the leaflet. The stroma is a moderately cellular, young, fibrous connective tissue, which shows no differentiation into special layers. The partitions dividing the blood-filled spaces

into several chambers are composed of stroma similar to that of the valves. Near the attachment of the valve to the myocardium a few blood vessels are inconstantly seen, though in the body of the leaflet no blood vessels are found (fig. 3).

#### ORIGIN

When we come to a consideration of the genesis of the nodules, we find a variety of opinions. Essentially there have been four expla-

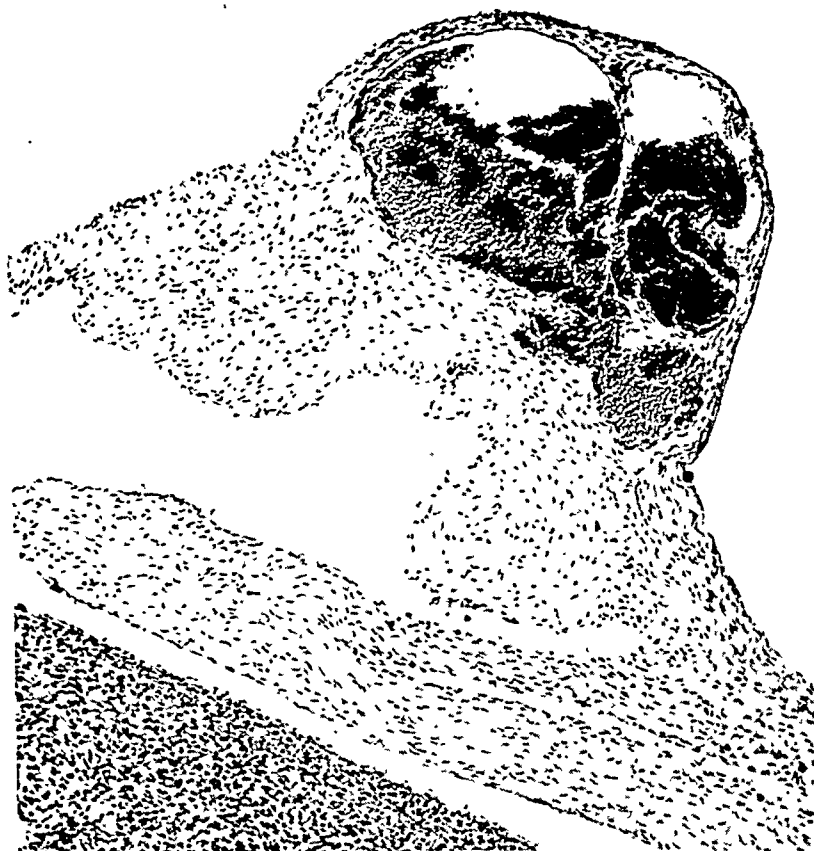


Fig. 2.—A microscopic section of a mitral valve, showing a blood cyst divided by a partition and filled with blood cells; hemalum and eosin stain;  $\times 40$ .

nations, each with minor modifications propounded by different observers:

1. The blood cysts represent extravasations of blood, namely, hematomas.
2. The cysts represent ectatic or dilated blood vessels.
3. The cysts represent angiomas.
4. The cysts are spaces filled with blood as a result of the pressure of blood into crevices running from the surface of the leaflet into the stroma forming the leaflet.

At first the nodules on the valve leaflets were considered as extravasations of blood, or hematomas. Elsässer in 1844 spoke of the extravasations. Luschka<sup>2</sup> cited Elsässer, and described the nodules, predominantly occurring on the auriculoventricular leaflets near to the free edge, as small extravasations of blood into the so-called nodules of Albini. Luschka assumed that the blood vessels which he was able to demonstrate were the source of the extravasations. Parrot<sup>3</sup> reported the occurrence of the blood nodules in 103 of 120 cases in which post-



Fig. 3.—A microscopic section of the same mitral valve as shown in figure 2, showing, in addition, endothelium-lined clefts extending into the stroma of the leaflet; hemalum and eosin stain;  $\times 40$ .

mortem examination was performed, and explained the origin of the nodules as the result of rupture of the vessels of the leaflets, and spoke of blood nodules rather than extravasations. Contrary to Luschka, who considered the extravasations as occurring into the nodules of Albini, Parrot believed that the blood nodules gave rise to the nodules of Albini. Von Kahlden<sup>9</sup> also adhered to the view that the hematomas on the valves represented bleeding into the connective tissue composing the

9. von Kahlden, C.: Beitr. z. path. Anat. u. z. allg. Path. **21**:288, 1897.

nodules of Albini. In this first explanation a mechanical traumatic factor was believed to be the significant initiating agent, with diapedesis or rhexis leading to the development of the hematomas.

The next view sought to emphasize vascular ectasias or varicosity as representing the essential feature of these nodules. Berti <sup>10</sup> emphasized that these nodules were blood-filled spaces lined by endothelium. He also noted that the nodules were not seen before the seventh month of intra-uterine life, and that they apparently disappeared after the fifth month of extra-uterine life. He explained the origin of the nodules as follows: In the vessels of the valves there is a gradually developing involutionary process which is accompanied by the proliferation of elastic tissue from the base to the margin, giving rise to constrictions and ectasias. Fahr <sup>4</sup> (with minor modifications) subscribed to the views of Berti, considering the nodules as vascular ectasias. By accepting the idea of vascular ectasia, both authors presupposed vessels to the valves. Fahr stated that vessels are found in early infancy and less frequently or not at all in adulthood. They occur more often in the auriculoventricular leaflets and hardly ever in the semilunar leaflets. Fahr, however, differed from Berti in considering the vascular regression as directly related to the disappearance rather than to the formation of the ectasias. He believed that the ectasias disappeared as a result of the progressive occlusion of the vascular network. As in the previously outlined explanation, mechanical traumatic moments were considered of significance.

While the view generally accepted was in the process of development, Nichols <sup>11</sup> reported the case of an infant with these blood cysts on the valves, and spoke of them as angiomas. This view was never extensively held and does not accord fully with all the facts demonstrated and recorded by the various observers. The cysts are neither newly formed blood vessels nor even dilatations of previously existing vessels.

The view now most widely acknowledged was developed in part by Haushalter and Thiry. They explained the origin of the blood-filled saccules as the result of the pressing of blood into small canals and emissaries by back pressure as a result of increased tension incidental to crying and exertion. The direction of the back pressure is toward the auricles. These canals that Haushalter and Thiry speak of have their origin at the marginal and submarginal insertion of the chordae tendineae into the leaflets of the valve on the ventricular surface. They found none of these nodules in stillborn infants, and considered this fact as confirmatory evidence in support of their view. Luschka,<sup>2</sup>

10. Berti, G.: München. med. Wchnschr. **45**:1194, 1898.

11. Nichols, J. L.: J. Exper. Med. **10**:368, 1908.

Berti,<sup>12</sup> Fahr<sup>4</sup> and others, however, found these nodules in stillborn infants from the sixth intra-uterine month to full term. Subsequent studies by various authors, however, led to amplification, some modification, and support of the ideas developed by Haushalter and Thiry.<sup>5</sup>

Thus Meinhardt<sup>13</sup> presented a report confirmatory in many respects of that of Haushalter and Thiry. He found that the auriculoventricular leaflets in the new-born infant are composed of a delicately meshed fibrous connective tissue stroma, rich in cells, indicative of the fact that active proliferation is yet taking place. The connective tissue fibers are delicate and without dominant arrangement into bands running in special directions. Furthermore, when one examines the mitral and tricuspid leaflets, the auricular side is uniformly smooth, but on the ventricular side the chordae tendineae lead to unevenness, clefts and indentations, lined by endothelium, leading into the valve substance. These clefts are not entirely simple, for they may divide, or give rise to ampulla-like dilatations. With systole blood is pressed into the clefts, and in diastole the blood flows out again, except when the blood is pressed into the narrower canals or into canals extending deep into the stroma of the leaflets. Thus are formed the cysts lined by the endothelium of the valve surface. In the case of the semilunar valves there are slight differences, but the principle is identical as outlined for the auriculoventricular valves. In the floor of the sinus of Valsalva, also, clefts enter into the substance of the leaflet and extend toward the ventricle. With the closure of the valves blood is pressed back into the sinus of Valsalva and into the clefts.

Wegelin<sup>6</sup> presented essentially the same observations as Meinhardt and Haushalter and Thiry. He stated that the pressure of blood into the endothelium-lined clefts by stretching and dilatation gives rise to cysts. The projection of the auriculoventricular nodules above the surface of the leaflet into the auricle and of the semilunar valve nodules into the ventricle was explained by the direction of the clefts and of the forces. By adhesion of the endothelium the clefts are closed, thus starting the process of obliteration of the canals which, when open, connect the lumen of the heart with the cysts. The canal being closed, the blood in the cyst becomes converted into a hyaline mass, and connective tissue ingrowth then completes the physiologic regression. The rarity of deeper indentations, their relatively infrequent occurrence, and the absence of chordae tendineae account for the lesser number of nodules in the leaflets of the semilunar valves. In older age the clefts are present, though in lesser number. Then, the valve tissue is denser and more resistant, hence the fact that the nodules are so rare in the adult

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12. Berti, G.: *Arch. f. Kinderh.* **31**:371, 1901.

13. Meinhardt, H.: *Virchows Arch. f. path. Anat.* **192**:521, 1908.

Bundschuh<sup>8</sup> further corroborated the work of Wegelin and the others holding similar views.

Jonsson<sup>7</sup> published a lengthy study confirmatory of the observations of Haushalter and Thiry, Wegelin, Meinhardt, Bundschuh and Hammes,<sup>14</sup> though disagreeing with the latter in speaking of the clefts as "vessels to the valve." Jonsson explained the monolocular cyst as a development from the multilocular cyst by thinning out and atrophy and eventual disappearance of the partitions as a result of the dilatation of the cyst. There are transitions. The monolocular cysts frequently contain rests of septums as small islets or as narrow bridges of tissue, identical with the stroma of the leaflet and lined by endothelium. Jonsson concluded that the clefts in the valve leaflets are to be considered as structurally analogous to the other irregular depressions in the inner surface of the heart. The cysts develop when blood is pressed into those spaces or potential spaces. That cysts develop depends on the specific structure of the leaflet as well as on the character of the tissue of the leaflet. In these studies of Jonsson and Wegelin reconstructions were used to demonstrate the connection of the cysts with the lumen of the heart.

Apparently there is no pathologic significance to these nodules. They are rather to be considered as a common anatomic finding.

#### SUMMARY

Blood-filled cysts, appearing as small, circumscribed, dark red, elevated nodules, are found on the valve leaflets of new-born infants.

The nodules are frequently found between the seventh month of intra-uterine life and the fifth month of extra-uterine life. In our series of infants the incidence was 75 per cent. After the first half year of life the frequency decreases to become a rarity after 2 years of age.

Four views relating to the genesis of the nodules are presented and discussed: 1. The nodules represent hematomas or extravasations of blood. 2. The nodules represent vascular ectasias. 3. The nodules represent angiomas. 4. The nodules represent endothelium-lined clefts into which blood is pressed and in which by dilatation cysts are formed. The last view is supported by wax reconstructions as well as by analysis of the demonstrable facts.

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14. Hammes, F.: Virchows Arch. f. path. Anat. **193**:238, 1908.



# PHEOCHROMOCYTOMA OF THE SUPRARENAL MEDULLA (PARAGANGLIOMA)

A CLINICOPATHOLOGIC STUDY

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Our purposes in this article are to collect all the recorded cases of pheochromocytoma of the suprarenal gland; to present the salient clinical and pathologic features of all these cases, and to attempt to reduce them to a common denominator, i. e., to present their clinicopathologic cross-section; and to report the fifth case of malignant pheochromocytoma of the suprarenal gland, one that is associated with another primary malignant growth (of the thyroid gland) and an unusual blood picture.

In many respects, the suprarenal glands are the most interesting organs in the body. Although they were discovered by Eustachius in 1563, their structure remained a mystery for over three hundred years, until von Kölliker described them histologically and until the first embryologic studies appeared in 1886.

So far as their physiology is concerned, although the early work of Vulpian, Czibulski and Szymonowicz, of Oliver and Schafer, of von Fuerth and particularly of Abel culminated in the brilliant isolation of epinephrine by Takamine, much is still unknown. While it is established that the medulla produces epinephrine, Hartman demonstrated the presence of the latter in the cortex as well, so that it is possible that, in addition to being "the most essential regulatory organ for cholesterol-fat metabolism" (Aschoff) and having something to do with secondary sexual characteristics, the cortex may be concerned in the production of epinephrine. We may say only that the suprarenal glands produce epinephrine, and that the suprarenal cortex is essential to life.

Of the greatest significance for the study of the tumors of the suprarenal glands, particularly those of the medulla, are the embryologic considerations, of which the most important is the fact that the suprarenal cortex and the medulla are practically two distinct and separate organs (as they are anatomically in the fish). While the anlage of the cortex

begins from coelomic epithelium, the first anlage of the medulla is formed by the migration of the sympathetic primitive cells (sympathogonia), which is usually complete when the embryo has reached the length of 85 mm. From the sympathetic primitive cells are developed the pheochromoblasts, and from these the mature medullary cells, or the pheochromocytes, which are large, polyhedral cells, each with a finely granular cytoplasm and a round vesicular nucleus, usually eccentric, containing nucleoli.

It is only natural, then, that dissimilar in their embryology, unlike in their histology and different in their physiology, the suprarenal cortex and the medulla give rise to tumors that are as distinct from each other as would be those of two different organs, pathologically just as much as clinically.

For a clearer understanding of the tumors of the medulla of the suprarenal gland, one must, therefore, remember that in the medulla three different types of tumors may arise, depending on the types of the cells from which they originate: (1) sympathogonioma (sympathoblastoma, neuroblastoma or neurocytoma), tumors derived from the embryonic sympathogonia; (2) ganglioneuroma, tumors derived from the mature ganglion cells, and (3) pheochromocytoma (chromaffin cell tumor, paraganglioma), tumors originating from mature pheochromocytes.

Since we are concerned only with the last-mentioned tumors, we must mention another general consideration concerning them before we pass to a thorough study of their pathologic and clinical manifestations: It is the question of the so-called chromaffin system. It was Kohn (1902) who suggested that this is a tissue system, pointing out that the cells of the so-called paraganglions—the carotid gland, the abdominal collection of cells and the so-called organ of Zuckerkandl (found at the bifurcation of the aorta, especially in children)—closely resemble the cells of the medulla of the suprarenal gland, not only in their morphology, but also in giving the so-called chromaffin reaction, i. e., the deposit of brown granules in the cells when the tissues are fixed in fluids containing potassium bichromate (owing to the fact that the latter, under the influence of epinephrine, is reduced to insoluble peroxide of chromium). Kohn (according to Bailey and Miller) also stressed the fact that paraganglions have a common embryonic origin with the medulla of the suprarenal gland, while Fulk and MacLeod furnished evidence that the active principle of the retroperitoneal chromaffin tissues has the same physiologic action as epinephrine. However, Maximow, pointing out that cells similar to the chromaffin tissue cells are also found in the kidney, liver, testis and heart, questioned the advisability of speaking of the “chromaffin system” and of including the medulla of the suprarenal gland in this system until “it has been shown that all of the chromaffin

organs have the same internal secretion," since "the assumption that the paraganglia elaborate epinephrine, just like the chromaffin cells of the medulla of the suprarenal, has not been securely established."

#### GENERAL FEATURES OF PHEOCHROMOCYTOMA

The first mention of this tumor is credited to Perley about 1890, although a case was mentioned by Fränkel in 1886. The first description is credited to Manasse in 1893. Fifty-three cases have been reported, all of which are tabulated elsewhere in this article.

Pheochromocytoma occurs in animals (Stilling; Zanfrotnini) as well as in man. In the former it is always benign, while in the latter it may be malignant, five undoubted malignant cases having been reported (Bonnamour, Doubrow and Montague; Gravier and Bernheim; Masson; King; Lazarus and Eisenberg) and one doubtful (Masson and Martin). All malignant cases have given widespread metastases.

*Incidence as to Age.*—Although cases have been reported in which the patients' ages ranged from 2½ to 82 years, the fifth decade of life is the one of greatest frequency of cases (about 30 per cent), with the fourth decade next (about 20 per cent). Three of the malignant cases occurred in patients 30, 47 and 68 years of age; the ages of the patients in the remaining two malignant cases are not known.

*Incidence as to Sex.*—The early impression that the tumor is much more prevalent among women than among men (according to Rabin, the ratio is three to one) is entirely erroneous. Of the forty-eight cases in which the sex of the patient was recorded, twenty-five occurred in women and twenty-three in men. Of the five malignant cases, two occurred in men and one in a woman; the sex of the other two patients is unknown.

*Distribution and Location.*—The question whether it is possible and advantageous to speak of the chromaffin system has been discussed; but regardless of the acceptance or nonacceptance of this concept, it must be admitted that pheochromocytoma not only occurs in locations other than the suprarenal glands, but is much more frequent in locations outside these glands, there being, according to Rabin, about eighty cases of pheochromocytoma of the carotid gland and three cases involving the organ of Zuckerkandl (Handschin; Hausmann and Getzowa; Stangl), one case of the retroperitoneal chromaffin tissue (Mayo) and one case of sacrococcygeal tissue (Alezaïs and Peyron).

As to the side on which the tumor occurs, in forty cases in which this information is available, the tumor occurred nineteen times on the right side and thirteen times on the left side, and in eight cases, the tumor was bilateral. All known cases of malignant pheochromocytoma (Alezaïs and Peyron, 1911) were bilateral.

*Association with Other Tumors.*—Neurofibromatosis (von Recklinghausen's Disease of the Skin): The following authors presented such combined cases: Herxheimer, Kawashima, Russum and Barry, Suzuki and Zeckwer—five cases in all (9.4 per cent).

Adenoma: Harbitz' patient had a cystadenoma of the pancreas (and a hypernephroma of the left kidney) as well as a pheochromocytoma of the right suprarenal gland. Hedinger's patient had an adenoma of the liver and a colloid adenoma of the thyroid gland, in addition to a pheochromocytoma of the left suprarenal gland.

Malignant Tumors of Other Organs: In one case (the second case of Lazarus and Eisenberg), a malignant pheochromocytoma was associated with a papillary adenocarcinoma of the thyroid gland; in another case (Harbitz), a benign pheochromocytoma was associated with a hypernephroma of the kidney on the opposite side (as well as with a benign cystadenoma of the pancreas).

Wahl's famous case was a combination of pheochromocytoma, ganglioneuroma and neurocytoma.

*Association with Hypertension.*—This probably is the most important condition coexisting with pheochromocytoma. Of fifty-three cases, twenty-five (47 per cent) showed the association with hypertension, while in six cases no information was recorded. Owing to the lack of clearcut abstracts of histories and necropsy protocols it is difficult to state just what the mutual relationships were. Did hypertension coexist with atherosclerosis? Did either one or the other or both coexist with renal disease? In what cases was hypertension continuous? In what cases was it paroxysmal? Was it present a sufficient number of years previous to the probable origin of the tumor to be definitely regarded as independent of the tumor?

The available data—fairly accurate—seem to give the following answers to these questions: Three patients showed diffuse atherosclerosis without renal disease and, so far as the information is available, without hypertension. Of twenty-five patients with hypertension, six had nephritis, three had diabetes, one was pregnant, and thirteen had pure (?) hypertension. So much for the statistics, but as soon as one attempts to rationalize and explain the available data, one is beset with great difficulties, some of which, in the light of present knowledge, are insurmountable.

To begin with, the first impression one forms from the study of the association of pheochromocytoma with hypertension is that, since it is the chromaffin tissue of the medulla of the suprarenal gland that produces epinephrine, and since epinephrine produces vasoconstriction and, *co ipso*, hypertension, it is natural to expect that an increase of the chromaffin tissue would bring about hyperadrenalemia and, *pari passu*,

hypertension. This conception of the causal connection between pheochromocytoma of the medulla of the suprarenal gland and hypertension is particularly alluring because pheochromocytomas located elsewhere, (e. g., those arising from the abdominal sympathetic ganglions, the carotid gland, etc.) have also been found to coexist with hypertension, which should be expected *a priori*, since it was pointed out by Fulk and MacLeod in 1916 that the retroperitoneal tissue contains an active principle with the same physiologic action as that of the suprarenal medulla—epinephrine. True, Fulk and MacLeod did not say that the active principle mentioned is epinephrine, but what they said means just that, for it is hardly possible to conceive that two organs of the same embryology and morphology could secrete two different active principles with the same physiologic action.

We now come to our first real difficulty. There have been at least twelve authentic cases of *cortical* tumors (benign and malignant) associated with hypertension (Oppenheimer and Fishberg; Rowntree and Ball). No explanation of such paradoxical findings has as yet been vouchsafed. If neoplasms of the chromaffin tissue (suprarenal and extra suprarenal) cause hypertension because of hyperadrenalemia (to be discussed presently), why should neoplasms of the suprarenal cortex—so different from the medulla embryologically, histologically and physiologically as to be quite properly regarded as a separate organ (as it is lower in the scale of vertebrate life)—cause hypertension? That they were primary cortical tumors is obvious from the fact that they were followed by characteristic changes in the secondary sexual characteristics. Again, in several cases, removal of the tumor led to disappearance of hypertension—*post hoc, ergo propter hoc*. On the other hand, if one considers how relatively frequent are renal hypernephromas, and how infrequent are suprarenal hypernephromas, and particularly, if one asks oneself why the infrequent cortical tumors in the suprarenal gland always cause a marked disturbance in the secondary sexual characteristics and frequently produce hypertension, while the frequent cortical tumors in the kidney do not, one grows perplexed in attempting to shed light on the connection between tumors of the suprarenal cortex and hypertension. Granting that the question why cortical tumors in the suprarenal gland cause hypertension and changes in the secondary sexual characteristics while such tumors in the kidney do not can be answered by stating that the renal hypernephromas are not cortical tumors but renal adenocarcinomas (an opinion subscribed to by many prominent pathologists), how can we explain the causation of hypertension by tumors of the suprarenal cortex if it is the suprarenal medulla that produces epinephrine (assuming, for the sake of the argument, that hypertension depends on hyperadrenalemia)? The fact that we may

be dealing in the case of the cortical tumor with hypertension of different causation from that in the case of tumor of chromaffin tissue—suprarenal and extrasuprarenal—seems to have been observed by Peyron, who pointed out that in the case of the cortical tumor hypertension is usually continuous, while in that of the chromaffin cell tumor hypertension is usually paroxysmal. To this we may add our own observation that in the case of malignant chromaffin cell tumor either hypertension is absent or there is actually hypotension. Peyron thought that the reason why hypertension is paroxysmal in the case of pheochromocytoma is that the intense proliferation and death of chromaffin cells interfere with the regular and complete secretion of epinephrine, and that, as a result of this, many proepinephrine and paraepinephrine particles get into the circulation, and thus, for the time being, there is no hypertension.

Attempts to account for the hypertension associated with cortical tumors are attended with difficulty, even if it is assumed that the suprarenal cortex produces epinephrine (Hartman), because, as Allen stated, "it has not been proved that epinephrine is necessary for the maintenance of normal vascular tone and blood pressure," and thus these attempts are at best few and highly speculative.

When we come to consider the relation of pheochromocytoma to hypertension, we still are confronted with two difficulties, namely, the inability to prove that hypertension is due to hyperadrenalemia, and the inability to demonstrate that hyperadrenalemia exists in such cases.

So far as the relation of hypertension to hyperadrenalemia is concerned, we wish to point out, as Maximow stated, that "it is quite probable that epinephrine never has physiologic function similar to its pharmacological action. . . . It is secreted in very minute quantities and is rapidly destroyed in the blood stream. Another view is that it is only an excretory product of protein metabolism, since it is probably destroyed before it reaches the arterial blood stream."

Stewart stated that "readers who peruse the literature without critical knowledge might easily conclude that epinephrine has a great functional importance. . . . Many writers assume this as self-evident. . . . The most convincing evidence that the epinephrine secretion does not play an indispensable rôle is the fact that the animals in which the epinephrine output has been suppressed . . . remain indefinitely in good health." He also stated that "it is frequently assumed . . . that the chromaffin inclusion of the adrenal medulla plays a rôle in the maintenance of the normal blood pressure. It has been measured in rabbits and dogs . . . before and during removal of the first adrenal, then up to and during removal of the second gland and then on till recovery or death. No change in the blood pressure was found which could be attributed to loss of the adrenals." Allen also

referred to the work of Ingier and Schmorl, who failed "to demonstrate increased amounts of epinephrine in the suprarenal glands of individuals with hypertension." Janeway, Mosenthal and many others regard the theory of hyperadrenalemia as a cause of hypertension as unproved.

The second difficulty referred to—that of the demonstration of hyperadrenalemia in patients with pheochromocytoma—remains insurmountable, since the present methods of estimating epinephrine in the blood are not accurate enough. We are therefore confronted with the fact that in some cases of tumor of the suprarenal cortex and in most of those of tumor of the medulla (as well as in those of tumor of extra-suprarenal chromaffin tissue) there is either paroxysmal-intermittent or continuous hypertension, and that very frequently removal of the tumor is followed by restoration of normal blood pressure. All explanations of any causal relationship between hyperplasia of the chromaffin tissue and hyperadrenalemia and between the latter and hypertension will remain unsettled so long as there is no positive information on the following points: Is epinephrine produced in the suprarenal medulla, in the cortex or in both? Is the active principle of the extrasuprarenal chromaffin tissue identical with epinephrine? Does the physiologic epinephrine in the human body act in the same way as the pharmacologic epinephrine? Does hyperadrenalemia exist in all the cases of pheochromocytoma? Does hyperadrenalemia mean hypertension?

*Association with Other Conditions.*—Tuberculosis: Five patients with pheochromocytoma suffered from tuberculosis; three of these had pulmonary phthisis, and two, a diffuse infection involving the lungs, intestines and lymph nodes. Manasse gave pulmonary tuberculosis as the cause of death in the case that he reported.

Diabetes: This was present in five cases—Herde's first case and those of Labbé, Tinel and Doumer, Biebl and Wichels, Helly and Schroeder; in one of the five cases, the pancreas was atrophied (Schroeder).

Vasomotor Instability: Six patients showed symptoms of shock; five of these died shortly after minor surgical procedures. Several other patients had attacks of dyspnea, pallor, numbness and, occasionally, unconsciousness.

Miscellaneous: In one case (Riemer), the patient presented an addisonian syndrome, and in another (Laignel-Lavastine and Aubertin) there was melanoderma.

#### PATHOLOGIC ANATOMY OF PHEOCHROMOCYTOMA OF THE SUPRARENAL MEDULLA

The tumor may be solid or cystic. In the latter case, the tumor is referred to as chromaffin medullary cystic struma. Of the fifty-three cases, eleven were cystic (two bilateral). All these were benign.

The size of the tumor varies from 1 cm. (Suzuki's second case [1909]; Herde's second case, on the right side) to 12.5 cm. (the first case of Lazarus and Eisenberg); several cases in which the tumor was from 9 to 10 cm. in length have been reported by Suzuki (third case, 1909), Hedinger, Oberling and Jung ("the size of a kidney"), Lascagna, Shipley and Schroeder. All the large tumors were benign. Two of the malignant tumors were 5 cm. long (Lazarus and Eisenberg; King). Information as to the length of the other three malignant tumors is not available. Speaking generally, the larger the tumor, the more apt it is to be cystic and hemorrhagic and to contain necrotic areas; for this reason, a large majority of the larger tumors are soft and reddish in appearance, while the smaller ones are pale gray.

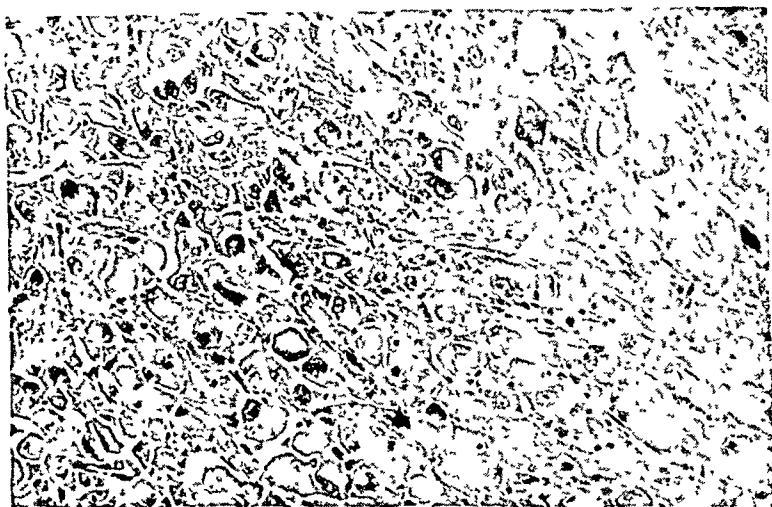


Fig. 1.—Section of malignant pheochromocytoma of the suprarenal medulla, showing the cellularity and the alveolar or trabecular appearance. Note the irregularity in size and shape of the cells. All the photomicrographs were made by A. Plato.

In some few cases, normal medulla could be recognized and was separated from the neoplasm by a distinct, at times very thick capsule, but in most of the cases, especially those of the larger growths, the entire suprarenal gland was converted into a tumor mass which obliterated the medulla but retained a thin, narrow rim of the cortex, usually interrupted at one or more points. At times, the solid part of the tumor occupies the central part of the mass, like the core of a hypernephroma, with multiple cystic areas all around it.

#### PATHOLOGIC HISTOLOGY OF PHEOCHROMOCYTOMA

The neoplasm (fig. 1) is very cellular and in many places has a slightly or definitely alveolar or trabecular appearance, depending on



the amount of the connective tissue stroma, which varies a great deal in different sections, but is always quite vascular, and envelops the cords of cells on all sides. Even with the lowest magnification, one is immediately struck with the extreme anisocytosis: not only are there occasional cells smaller than erythrocytes and others more than twice as large as neutrophil leukocytes, but less striking differences in size are so frequent and widespread that one's impression is almost kaleidoscopic, for it seems as though there are no two cells of the same size. This feature becomes the more striking the greater the magnification. The great majority of the cells are large, about from 30 to 35 microns long. In form, most of the cells are polyhedral, oval or irregularly trapezoid. At times, one sees a cell strongly resembling the histiocyte as seen in blood films, an ovoid cell, from 50 to 75 microns long, with a very pale cytoplasm and a centrally placed, dark round nucleus, about 20 microns long, containing many nucleoli.

The cytoplasm of most of the cells is abundant and is very finely granular, although a dull, homogeneous appearance is not at all lacking in many cells. Sections stained by the hematoxylin-eosin method show that most of the cells are distinctly acidophil, and that cells deeply stained with hematoxylin are exceptional. Sections fixed in bichromate solutions show brownish granules in the cytoplasm. A few contain large, round, hyaline bodies, occasionally containing dark, rodlike structures.

The nuclei (fig. 2) show some characteristic features. In the first place, they vary in size as much as do the cells themselves: occasionally a nucleus is not more than from 5 to 8 microns in diameter; most frequently it is from 15 to 30 microns long and, not rarely, much larger, depending on the size of the cell. Its location is most frequently eccentric, and in many cells its flattened appearance along a cytoplasmic edge gives the cell a striking resemblance to the "signet ring" cell of a Krukenberg tumor. A syncytial arrangement is not at all rare. One notices also that, occasionally, the nucleus is so pyknotic as to resemble a mere nuclear fragment. The shape of the nucleus is highly irregular: most frequently it is oval or cylindric, irregularly round or flattened out; occasionally it is almost triangular; not infrequently it is so highly bizarre as to be indescribable architecturally. Many cells are multinuclear, and from time to time one sees a cell in which the nucleus is very large and resembles a ball of twisted worsted. The staining of the nucleus is also irregular, but in an overwhelming majority of the cells the nucleus is always distinctly hyperchromatic. Even here, however, the staining is never uniform, the different parts of the nucleus staining with varying intensity. The chromatin network is usually coarse and in many cells irregularly twisted. In some cells the nucleus

is vesicular and has a "washed-out" appearance. This is particularly frequent in multinucleated cells. In most cells, the nucleus contains several nucleoli and, at times, very dark, irregularly shaped granules. The presence of fat or glycogen has not been established. In malignant tumors, mitotic figures are frequent, as are chromatin-rich nuclei (embryonic cells?). To sum up the appearances of the cells and of their nuclei, we should say that the most striking feature is the irregularity of size, shape and staining properties. Peyron regarded the

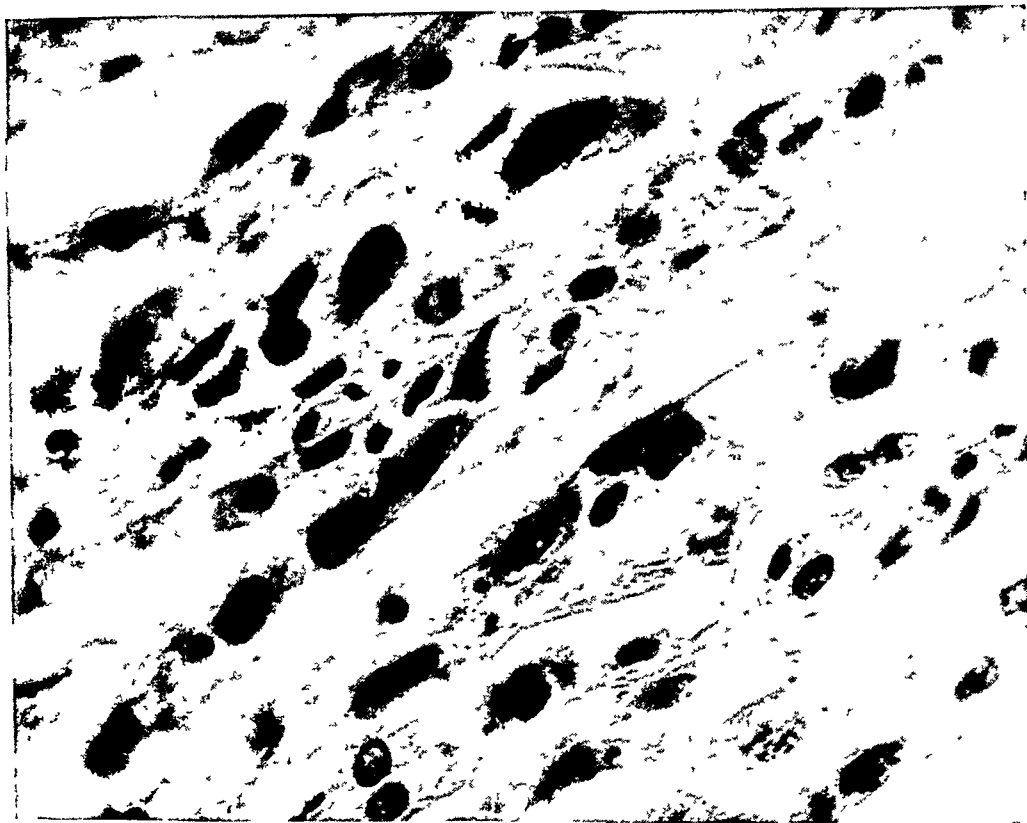


Fig. 2.—High power magnification of the tumor tissue, showing the variation in size, shape and position of the nuclei; reduced from  $\times 340$ .

formation of irregular cellular masses through invasion of the fibrous septums as an early sign of malignancy.

It has been mentioned that these tumors are rich in capillaries. These are, as pointed out by Alezais and Peyron, separated from the tumor tissue by vascular endothelium. Of the vascular spaces there are two types: those in contact with the external surfaces of the epithelial cords and containing erythrocytes and largely neutrophil leukocytes, and the intra-epithelial capillaries, the meshes of which are perpendicular to the capillaries of the first type and contain cloudy, grumous material.

## CHEMISTRY OF PHEOCHROMOCYTOMA

Qualitative demonstrations of the presence of epinephrine in the tumor were done in several cases, but the quantitative determination was carried out only by Rabin, who found the amount of epinephrine much larger than the normal. Since there is no method of demonstrating hyperadrenalemia, its relation to hypertension remains unsettled.

## REPORT OF A CASE

This is the second case in the article by Lazarus and Eisenberg. The case was only mentioned in that article. Because of its many unusual features, such as its malignancy, its association with another primary malignant neoplasm of the thyroid gland, unusual places of metastasis—e. g., the gum, etc.—the complete description and the discussion of the case have been reserved for this article, dealing as it does with a complete study of all pheochromocytomas reported in the world's literature.

*Clinical History.*—A Russian Jewess, aged 63, was admitted to Sydenham Hospital on Jan. 1, 1930, complaining of a painful mass on each side of the neck and a small growth on the left lower gum. The latter growth had reached the size of a large pea three weeks after the extraction of the left lower bicuspid. Ten years before examination, she had noticed a slight swelling in the right submaxillary space. About four years before examination, she had noticed that her neck was getting larger; one year before examination, this swelling had caused her neck to become quite large. She refused surgical relief. About three months before examination, she began experiencing fairly constant backache and transient headache, and during the last three months had lost 25 pounds (11.3 Kg.) in weight, as well as noticed nodular masses on each side of her neck. A reference has already been made to the extraction of a tooth, promptly followed by the appearance on the gum of a reddish growth, the size of a large pea.

Physical examination revealed a sallow woman about 60 years of age, cachectic-looking and greatly depressed. The growth in the gum was tender and friable and bled easily. The thyroid gland felt hard and was irregularly and considerably enlarged. Bilateral inguinal as well as cervical adenopathy was present. Percussion sounds varied from dulness to flatness over the bases of the lungs and the left upper lobe, and there were coarse râles and tubular breathing over these areas. Examination showed nothing else that was significant, except pain over the dorsal and lumbar vertebrae.

Roentgenologic examination revealed metastatic nodules in both lungs.

All the laboratory data were negative, except the results of examinations of the blood, which were, so far as we have been able to ascertain, unique in cases of pheochromocytoma. The patient came into the hospital with a total white cell count of 22,000 and the following differential count: segmented neutrophils, 60 per cent; nonsegmented neutrophils, 10 per cent; monocytes, 5 per cent; lymphocytes, 6 per cent; basophils, 1 per cent, and eosinophils, 18 per cent. She also had moderate secondary anemia. Gradually the total count rose to 63,000, with the nonsegmented (band) neutrophils constituting 50 per cent; the lymphopenia persisted, and the eosinophilia persisted during the entire course, ranging between 10 and 25 per cent.

Another important feature was the absence of hypertension, the blood pressure ranging between 130 systolic and 85 diastolic and 120 systolic and 75 diastolic.

The patient's progress was steadily downward, and on Feb. 27, 1930, she died.

*Résumé of Postmortem Examination.*—The left side of the mouth was filled with a fungating, necrotic mass. Several large, discrete, firm lymph nodes were felt on each side of the neck.

The thyroid gland was not recognizable as such, there being in its place a piece of homogeneous, soft tissue, the size of a tangerine, encapsulated, but with the capsule perforated and invaded in several places. On section, the tissue was grayish yellow and contained numerous hemorrhagic and necrotic spots.



Fig. 3—Pheochromocytoma. *A*, primary growth in the suprarenal glands, with kidneys normal; *B*, metastasis to the lung; *C*, metastasis to the intestines; *D*, metastasis to the mesenteric glands.

When the chest was opened, a necrotic mass was found in the upper lobe of the left lung, the size of an orange, adherent anteriorly to the five upper ribs, and a smaller mass in the lower lobe, anteriorly. The right lung showed an entire lobe converted into a similar necrotic mass (fig. 3 *B*).

The liver, normal in size, contained a few superficial metastatic foci.

The spleen, fully twice as large as the normal, showed the lower half converted into homogeneous, grayish, necrotic tissue.

Both kidneys were normal.

Both suprarenal glands were destroyed and replaced by two masses of soft, grayish, necrotic tissue, each with just a thin rim of the cortex. These masses were twice the size of the normal glands (fig. 3 *A*).

Author	Patient		Symptoms	Gross Appearances	Histologic Appearances
	Sex	Age			
Barker.....	M	47	Weakness, dyspnea, hypertension, epileptiform attacks, for 6 months	Cystic, broken-down tumor of left suprarenal medulla; generalized arteriosclerotic lesions in brain; hemorrhage in peduncles, pons and fourth ventricle; no metastases	Immature chromaffin cell tumor
Berdez.....	M	70	Died of pneumonia.....	Brownish-red tumor, 3 by 2 cm.....	Cellular tumor; groups of cells separated by capillary nets, some of which are large enough to occupy one half the visual field; cells resemble medullary cells
Bergstrand.....	F	40	Hypertension .....	Tumor of right suprarenal.....	Paraganglioma
Bieble and Wiehels.....	M	36	Hypertension, diabetes, sclerotic kidneys, traumatic loss of eye (war), dizziness, pseudobulbar paralysis, sudden unconsciousness and death	Bilateral suprarenal tumors; left, size of hen's egg; right, plum size; tumors dark grayish-red; basal vessels sclerotic; foci of apoplectic softening in basal ganglions; frank pontile bleeding; no metastases	Chromaffin cell tumor
Bonnamour, Doubrow and Montague	?	?	Malignant paraganglioma with metastases to lymph nodes and pleura; in the latter, the growth differed from that in the former in that it contained no blood vessels and the cells were much less differentiated		
Connor.....	{ M M F	{ 40 to 50	Benign tumors associated with hypertension		
Fränkel.....	F	18	Scarlatina at 14; hypertension, vomiting and headaches	Bilateral suprarenal tumor; right, size of nut; left, size of fist; both give chromaffin reaction	Called angiosarcoma, but probably paraganglioma
Gravner and Bernheim.....	?	?	Blood pressure 105/70.....	Bilateral malignant tumor with metastases to mediastinum	
Harbitz.....	M	47	.....	Hypernephroma of left kidney with metastases; cystadenoma of pancreas; right kidney shows metastases from left, and a tumor of suprarenal	Paraganglioma
Hellinger.....	F	34	Died a few days after abortion; general clinical symptoms not clear	Left suprarenal has mass (10 cm.), encapsulated, adherent to diaphragm and pancreas; largely cystic, with shreddy tissue in the wall; no metastases	Cystic paraganglioma (struma medullaris cystica suprarenalis); positive chromaffin reaction
Helly.....	M	43	Hypertension, glycosuria.....	Tumor of right suprarenal, size of apple....	Paraganglioma
Herde.....	F	62	Glycosuria, arteriosclerosis .....	Tumor of right suprarenal, 7 by 6 by 5 cm.; few small, reddish-gray cysts	Paraganglioma
Hertz.....	F	45	Hypertension .....	Bilateral suprarenal tumor; left, 3 cm. in cross-section, grayish-red; right, 1 cm., with few cystic areas; cardiac hypertrophy	Bilateral paraganglioma; frequent giant cells; much glycogen in left tumor
Hirxhelmer.....	M	55	Neurofibromatosis .....	Tumor of right suprarenal.....	Paraganglioma
Kawashima.....	F	33	Adnexal disease; macerated fetus; no hypertension; neurofibromatosis of skin	Tumor of left suprarenal.....	Primary hyperplasia of medullary cells, apparently paraganglioma

Kerpola.....	?	?	Hypertension .....	Tumor of suprarenal medulla.....	Paranganglioma
King.....	M	30	Loss of weight for 12 months; pain in joints; operated on for goiter 4 years previously; several subcutaneous nodules on chest and abdomen	Bilateral suprarenal tumor; left, 5 by 2 cm.; right, smaller; metastases to liver, lungs, bones, brain; chromaffin reaction positive	Malignant paranganglioma
Labbé, Azerad and Violle	M	29	Hypertensive crises (pallor, anguish, tachycardia, etc.)	Chronic glomerulonephritis; right suprarenal weighs 120 Gm.	Paranganglioma
Labbé, Thinel and Doumer	F	28	Diabetes; vomiting attacks 3 to 4 times a week; puromysmal hypertension, 260/180 to 150/100	Kidneys normal; tumor of left suprarenal	Paranganglioma
Lalaguel-Lavastine and Aubertin	M	35	Chronic tuberculosis, melanoderma.....	Whitish nodule, size of pea, in right suprarenal	Medullary adenoma
Lascagna.....	M	61	Unable to work; cough for eight months	Bilateral suprarenal tumor, each gland weighing 55 Gm.; left tumor 9 by 6 by 2.5 cm.; right, 9 by 4.5 by 3 cm.	Paranganglioma (?) , general structure showing giant cells, mosaic appearance, richness of capillaries; positive chromaffin reaction; no metastases
Lazarus and Eisenberg	F	58	Hematuria, pyuria and pain in left lumbar region; progressive loss in strength.	Ovoid tumor of left suprarenal, 12.5 by 8.5 by 6 cm., cystic, filled with clotted blood and pseudomucinous material, rimmed with cortex	Pheochromocytoma
	F	62	Progressive enlargement of thyroid; nodule on left lower gum following extraction of tooth; loss of weight and strength; mass in right renal region	Bilateral suprarenal tumor; metastases to liver, lungs, bones, intestines, glands and gum	Malignant pheochromocytoma
Manasse.....	F	Old	Died from pulmonary tuberculosis.....	Tumor of left suprarenal, size of hen's egg	Paranganglioma
	M	64	No information .....	Tumor of left suprarenal, size of hen's egg	Paranganglioma
Marchetti.....	M	15	Bilateral tumor (10 and 11 Gm.), apparently Bruchanow, Dagonet, etc.	Apparently paranganglioma and ganglioneuroma, as he says his case resembles that of	
Masson.....	?	?	.....	.....	Paranganglioma
	?	?	.....	Metastases to lymph nodes and liver.....	Malignant paranganglioma
Masson and Martin.....	F	45	Cachexia; pain in right side of abdomen for few months; died 6 hours after operation	No autopsy; only small piece of tissue available	Malignant paranganglioma (?)
Mayo.....	F	30	Hypertension, headache, vomiting.....	Tumor of left suprarenal medulla.....	Paranganglioma
Neusser and Wiesel.....	?	?	Marked arteriosclerosis.....	.....	Chromaffin cell tumor
	?	?	Vasomotor instability; died 2 hours after extraction of tooth	Bilateral suprarenal tumor, cystic.....	Chromaffin cell tumor
Oberling and Jung.....	F	28	Pregnancy; blood pressure 250/190; after delivery, patient turned pale, with pulse 150, and died in 6 hours	Tumor of right suprarenal, size and shape of kidney	Paranganglioma
Orth.....	M	47	Hypertension, renal disease.....	Tumor of right suprarenal, size of duck's egg	Paranganglioma
Perley.....	M	70	.....	Tumor of left suprarenal.....	Paranganglioma
Rabin.....	F	45	Chronic nephritis, hypertension, exophthalmic goiter	Tumor of right suprarenal, 4 cm.....	Pheochromocytoma

*Cases of Pheochromocytoma Collected from the Literature—Continued*

Author	Patient		Symptoms	Gross Appearance	Histologic Appearance
	Sex	Age			
Riener.....	F	46	Addisonian syndrome .....	Tumor of right suprarenal, cystic.....	Paraganglioma
Robert.....	M	46	Hypertension; right lumbar tumor.....	Mass involving right suprarenal and kidney, weighing 700 Gm.	Paraganglioma
Russum and Barry.....	M	56	Neurofibromatosis; large mass in right upper quadrant of abdomen	Neurofibromatosis of skin, urinary bladder and mediastinum; right "polymorphous cell peripheral sarcoma, probably derived from a neurofibroma"; left paraganglioma; multiple metastases from first tumor	Paraganglioma
Schrouder.....	F	42	Hypertension, diabetes .....	Arteriosclerosis; cardiac hypertrophy; atrophy of pancreas; bilateral suprarenal medullary tumor; right, size of small apple; left, size of fist	Paraganglioma
Shibley.....	F	26	Hypertension .....	Tumor of medulla of right suprarenal, 9 by 7 by 3.5 cm., with small cysts and hemorrhagic areas	Paraganglioma
Suzuki.....	F	60	Neurofibromatosis, pulmonary tuberculosis	Pulmonary, intestinal, left suprarenal and pelvic peritoneal tuberculosis; medulla of right suprarenal replaced by grayish-yellow nodule	Paraganglioma
	F	82	Pneumonia on right side, emphysema, chronic bronchitis	Arteriosclerosis; tuberculosis of lungs and lymph nodes, etc.; tumor of right suprarenal	Paraganglioma and lipoma; partial chromaffin reaction
Thomson.....	M	62	Sepsis, fractured femur, bronchopneumonia on right side	Tumor of left suprarenal, 10 cm., necrotic and cystic	Paraganglioma
	F	80	Irrelevant .....	Tumor of left suprarenal, 4.5 by 2 by 4 cm., cystic, reddish-gray; no metastases	Paraganglioma
Vaquez, Donzelot and Gerudel	M	37	Transient hypertensive attacks, with pain (from lower extremities to the head—no vomiting, no convulsions)	Tumor of right suprarenal, 7 by 6.5 cm.	Paraganglioma
Wahl.....	F	2½	.....	Malignant tumor with metastases.....	Neuroblastoma, ganglioneuroma and paraganglioma
Weber.....	F	40	Hypertension .....	Cystic mass; no histologic examination....	Paraganglioma (?)
Wegelin.....	F	39	Pulmonary tuberculosis .....	Chronic tuberculosis of lungs, intestines and mesenteric glands; sclerotic kidneys; tumor of right suprarenal, 5 cm., soft, grayish-red	Paraganglioma
Wiehels and Biehl.....	M	33	Hypertension; contracted kidneys.....	Tumor of right suprarenal, size of hen's egg	Paraganglioma
Zeekwer.....	F	36	Paralysis of right arm and leg 3 months prior to admission; positive Wassermann reaction; attacks of unconsciousness	Softening of right internal capsule; aortic syphilis, grayish-red tumor of medulla of left suprarenal, 4 by 3 by 2.5 cm.; neurofibromatosis of spinal nerves; no metastases	Chromaffin cell tumor; no metastases; no invasion of structures outside of suprarenal

The small intestine (fig. 3 C) showed in several places, scattered throughout the jejunum and the ileum, nodular, pale, necrotic masses varying in size from that of a pea to that of a cherry. In several places these growths, located in the intestinal mucosa, had perforated and had caused adhesions between adjacent loops. Several mesenteric glands (fig. 3 D), ranging in size from that of a cherry to that of a tangerine, were found. The left psoas muscle showed, in its middle third, a soft, necrotic growth, the size of a plum. The five upper ribs in the axillary line on the left side were converted, for a distance of 8 cm., into soft, necrotic tissue, as were the bodies of the sixth, seventh, eighth, ninth and tenth dorsal vertebrae.

Examination revealed nothing else that was significant, except moderate myocardial fibrosis and atherosclerosis.

*Microscopic Examination.*—The tumor of the thyroid gland was a papillary adenocarcinoma. No metastatic growths from this could be discovered. All other neoplastic tissues described in the separate organs were alike, none of them showing any resemblance to the carcinoma of the thyroid gland.

Sections from these tissues, taken from the suprarenal glands, the lungs, the bones, the small intestine, the mesenteric nodes, the spleen and the liver, and a biopsy specimen of gum showed the following uniform picture: a very cellular growth, very richly vascular, with abundant thin connective tissue septums, giving the tumor a trabecular, alveolar appearance. Numerous capillaries lay in close contact with the cellular cords.

The striking feature was the extreme irregularity in the size and the shape of both the cells and the nuclei, as well as in the location of the latter in relation to the cytoplasm. The cells showed finely granular cytoplasm, and in sections fixed in bichromate solution numerous brown granules were seen—the chromaffin reaction. The cells varied in size tremendously, being from 15 to over 75 microns long, were most frequently polyhedral or ovoid, and were acidophil. Here and there were monstrosly large cells resembling histiocytes. Homogeneous, hyaline bodies were observed in some cells.

The nuclei also varied greatly, some being pyknotic and hyperchromatic, others vesicular and pale, containing one or more nucleoli and, occasionally, black, rod-like granules. Their location was, for the most part, eccentric, a few cells showing the nucleus flattened out at one edge, the cells then resembling the "signet ring" cells of a Krukenberg tumor. A syncytial arrangement was frequent.

No glycogen or fat could be demonstrated by special staining methods.

*Pathologic Diagnosis.*—Bilateral malignant pheochromocytoma of the suprarenal gland, with metastases to the mouth (gum), lungs, liver, spleen, left psoas muscle, mesenteric glands, small intestine (with multiple perforations), sixth to tenth dorsal vertebrae and five upper left ribs; papillary adenocarcinoma of the thyroid gland, without metastases; fibrous myocarditis, and moderate diffuse atherosclerosis.

*Summary.*—A Russian Jewess, aged 63, with a history of enlargement of the neck in the region of the thyroid gland over a period of ten years, showed, on admission to the hospital, large lymph nodes on both sides of the neck and a small growth on the left gum. She had lost 25 pounds (11.3 Kg.) in weight during the previous three weeks. Roentgenologically there were metastatic nodules in both lungs. She had a high leukocyte count (60,000), which gradually came down to normal, and persistent eosinophilia (from 10 to 25 per cent). The blood pressure was normal at all times. She died three weeks after her admission to the hospital. The autopsy revealed, in addition to moderate generalized atherosclerosis and fibrous myocarditis, a papillary adenocarcinoma of the thyroid gland without



metastases, and a malignant bilateral suprarenal pheochromocytoma with metastases to numerous viscera and bones. Death was undoubtedly due to multiple necroses and exhaustion.

#### REVIEW OF CASES REPORTED IN THE LITERATURE

The reader is referred to the accompanying table containing the names of the authors who have reported cases, and age and sex of the patient and the main clinical and pathologic features in each case.

#### SUMMARY

Fifty-three cases of pheochromocytoma of the medulla of the suprarenal gland collected from the literature are tabulated with their main clinical and pathologic features.

A new case of malignant pheochromocytoma of the medulla of the suprarenal gland is presented, with the following unusual features: (*a*) coexistence of another primary malignant neoplasm (of the thyroid gland), (*b*) widely spread metastases from the pheochromocytoma, with none from the carcinoma of the thyroid gland, and (*c*) a very unusual blood picture.

Only five of the fifty-three cases previously reported were malignant, all with widespread metastases.

Association of benign pheochromocytoma with other tumors is not rare, several cases having been reported of its association with multiple neurofibromas, with adenomas (of the thyroid gland, liver and pancreas) and with hypernephroma.

In only one case was a malignant pheochromocytoma associated with another primary malignant tumor.

All known cases of malignant suprarenal pheochromocytoma were bilateral.

The incidence of the tumor is about even as to sex; it is greatest on the right side and in patients in the fifth decade of life.

The most striking histologic features are: the greatest imaginable irregularity in the size and shape of the cells and of the nuclei, the chromaffin reaction and the rich vasculature.

The most interesting feature is the relation of these tumors to hypertension. About one half of all the patients showed hypertension, some with atherosclerosis and others without it. While in some cases of tumor of the suprarenal cortex hypertension was continuous, in a large majority of the cases of tumors of the suprarenal medulla in which it was present it was of paroxysmal type—but only in the cases in which the tumor was benign, as all patients with malignant medullary tumor showed either no hypertension at all or hypotension.

The attempts to correlate hyperplasia of chromaffin tissue with hypertension are not successful because of the absence of proof that

hyperadrenalemia exists in such cases, and also because it is not at all established that hyperadrenalemia is responsible for hypertension.

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# JAMES BRYCE AND HIS TEST FOR PERFECT VACCINATION

A FORGOTTEN CHAPTER IN THE HISTORY OF IMMUNOLOGY

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CHICAGO

## I

Long before the modern study of allergy, modified forms of small-pox ("varioid") and of vaccinia ("vaccinoid") were explained by the previous action on the body of the variolous or vaccinal virus. In the modern phrase, these modified forms, as well as certain related phenomena, were interpreted as manifestations of specific sensitization. The earliest description of the allergic relationship between smallpox and vaccinia is given by Jenner in a footnote to case 4 in his "Inquiry":<sup>1</sup>

Case IV. Mary Barge, of Woodford, in this parish was inoculated with variolous matter in the year 1791. An efflorescence of a palish red colour soon appeared about the parts where the matter was inserted, and spread itself rather extensively, but died away in a few days without producing any variolous symptoms. She has since been repeatedly employed as a nurse to Small-pox patients, without experiencing any ill consequences. This woman had the Cow Pox when she lived in the service of a Farmer in this parish thirty-one years before.

The footnote reads as follows:

It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces the Small Pox. Indeed it becomes almost a criterion by which we can determine whether the infection will be received or not. It seems as if a change, which endures through life, had been produced in the action, or disposition to action, in the vessels of the skin; and it is remarkable too, that whether this change has been effected by the Small Pox, or the Cow Pox, that the disposition to sudden cuticular inflammation is the same on the application of variolous matter.

Instances more or less like the one described by Jenner in Mary Barge are described in the same year by George Pearson in his book about cowpox:

A man, 26 years, had cowpox six years previously, inoculated with smallpox. On the fifth day the appearance was like that of a gnat bite and it was thought that the inflammation seemed to be too rapid for that of variolous infection when it produces the smallpox.

In another case inoculation with smallpox virus of a young man who had cowpox ten years previously resulted in inflammation that was judged to be premature with respect to smallpox. . . .<sup>2a</sup>

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From the John McCormick Institute for Infectious Diseases.

1. Jenner, Edward: *An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucester, and Known by the Name of the Cow Pox*, London, S. Low, 1798.

2. Pearson, George: *An Inquiry Concerning the History of the Cowpox, Principally with a View to Supersede and Extinguish the Smallpox*, London, J. Johnson, 1798, (a) p. 15; (b) p. 34.

A servant girl had the cowpox early in life and years later she was inoculated, "but nothing took place, except the appearance of a red blush round the incision. . . ." <sup>2b</sup>

Jenner's remarkable statement, a pioneer record of an allergic phenomenon, apparently escaped the notice of writers and observers for more than one hundred years—a telling example of the truth in Benjamin Ward Richardson's remark about Jenner's "Inquiry": "No book so small, has been talked of so much; no book has been read from the original so little; no book of such dimensions has made the name of any author so famous." Von Pirquet,<sup>3</sup> for instance, made his elaborate study of vaccinal allergy without knowledge of Jenner's and later descriptions of the phenomenon or of the work in Jenner's time and subsequently on its practical value. For no sooner had Jenner described vaccination against smallpox than efforts began to find a test for the perfect vaccination. In most cases vaccination gave rise to a local lesion without marked constitutional symptoms. It was feared that it might act locally without inducing the desired general action. How was one to be assured that so mild a process would protect against smallpox? To use Jenner's conclusive test, namely inoculation of the vaccinated with smallpox matter, was impractical and dangerous.

John Pearson writes: <sup>4</sup>

. . . since Vaccine produced but little disorder of the constitution, and is not attended by an eruption on any part of the body, except that to which the infectious fluid is applied, it would be very desirable to have some criterion by which we could be assured that the vaccinated person has undergone that inexplicable change which secures him against the Small Pox.

Before long the allergic reaction of the skin to vaccine virus was used as such a criterion. The idea appears to have arisen in the minds of several vaccinators at the same time. Was perhaps Jenner's footnote the common source even if it is not mentioned? The leader in this now forgotten search for a simple test for perfect vaccination was James Bryce of Edinburgh.

In 1801, the *Annals of Medicine* printed this note:

Mr. Bryce, one of the Surgeons who inoculate the cow-pock at the Public Dispensary of Edinburgh, has been for some time engaged in making experiments, with a view to ascertaining some important particular in the history of that disease, the results of which he will communicate to the public.<sup>5</sup>

The outcome of these experiments was published in a book in 1802, four years after Jenner's "Inquiry." A second, enlarged edition was published in 1809 (figs. 1 and 2).

3. von Pirquet, C.: Wien. klin. Wchnschr. **19**:885 and 1407, 1906; Klinische Studien über Vakzination und vakzinale Allergie, Leipzig, F. Deuticke, 1907; Arch. Int. Med. **7**:259, 1911.

4. Pearson, John: General Observations on the Cow-Pox, in Willan, Robert: Vaccine Inoculation, London, R. Phillips, 1806, appendix, p. IX.

5. Ann. Med. **6**:486, 1801.

In this book, which is quoted frequently in the writings on vaccination, especially during the next forty or fifty years, Bryce discusses the general history of cowpox, the “advantages to society from the general practice of inoculation for cowpox,” the “circumstances to be

PRACTICAL OBSERVATIONS

ON THE

INOCULATION

OF

C O W P O X.

POINTING OUT A TEST OF A CONSTITUTIONAL AFFECTION IN THOSE CASES IN WHICH THE LOCAL INFLAMMATION IS SLIGHT, AND IN WHICH NO FEVER IS PERCEPTIBLE.

ILLUSTRATED BY CASES AND PLATES.

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By JAMES BRYCE,

MEMBER OF THE ROYAL COLLEGE OF SURGEONS, EDINBURGH, SURGEON TO THE ORPHAN HOSPITAL, AND ONE OF THE SURGEONS TO THE INSTITUTION FOR THE GRATUITOUS INOCULATION OF COWPOX.

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Edinburgh:

PRINTED FOR WILLIAM CREECH; AND T. CADELL, JUN.

& W. DAVIES, LONDON.

1802.

AND SOLD, EVERYWHERE.

Fig. 1.—Title-page of book by James Bryce on inoculation of cowpox, published in 1802.

carefully attended by those conducting the inoculation of cowpox,” and the medical treatment of cowpox. I am concerned here especially with his “test of a constitutional affection in those cases in which the local inflammation is slight, and in which no fever is perceptible” or, as he has it on the title-page of the second edition, “a certain test of perfect vaccination.”

In the course of his study of cowpox inoculation, he recollected

. . . some experiments which had been made with regard to the inoculation of smallpox. It was found, that if the same person was inoculated every day until the fever induced by the first inoculation supervened, all the other punctures

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PERFECT VACCINATION.

*Illustrated by Cases and Plates.*

THE SECOND EDITION.

WITH AN

### APPENDIX,

CONTAINING ADDITIONAL OBSERVATIONS, TOGETHER WITH  
A PLAN FOR EXTINGUISHING THE CONTAGION OF THE  
SMALLPOX IN THE BRITISH EMPIRE, AND FOR  
RENDERING THE VACCINE INOCULATION GE-  
NERAL AND EFFECTUAL.

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BY JAMES BRYCE, F.R.S. EDIN.

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*Alex. Smellie, Printer.*

1809.

Fig. 2.—Title-page of second edition of James Bryce's book published in 1809.

quickly advanced in their progress; and that, in the course of a day from the time the fever or general affection began, even that puncture which had been last made, perhaps only twenty-four hours before, equalled in maturity the one first made, perhaps eight or nine days before, and from which the fever had arisen.<sup>6a</sup>

6. Bryce, James: *Practical Observations on the Inoculation of Cowpox*, ed. 1, Edinburgh, W. Creech, 1802, (a) p. 173; (b) p. 175; (c) p. 189; (d) p. 214.

Here Bryce probably had in mind the experiments of Woodville, who wrote:<sup>7</sup>

Thus if a person be alternately inoculated with variolous matter, and with that of the Cow-pox every day till the fever is excited, all the inoculations make a progress; and as soon as the whole system becomes disordered, they appear to be all equally advanced in maturation.

Bryce reaches the significant conclusion that because the pustule caused by the last puncture suddenly reached maturity at the time of the general or constitutional affection, "this alone was a sufficient proof of the presence of the variolous action in the system." He reasons that the same sort of reaction would occur in cowpox or vaccinia. The events in probably accidental inoculation in the course of vaccination supported him in this view:

And certainly, if we find in cowpox, where the inflamed and hard areola does not take place, at least in the regular course of that affection, until the end of the seventh or beginning of the eighth day from inoculation, that a second inoculation, performed for example at the end of the fifth or beginning of the sixth day, is so much accelerated in its progress, about the time the general affection of the system usually takes place, as to have an areola formed within a few hours, or very shortly after the first, and that this areola increases with the first, and again fades at nearly the same time, we must be struck with the similarity and be forcibly led to draw the same conclusion in this case as in the former, respecting the smallpox, viz., that although the inoculated affection had appeared very slight, and no fever had been observed, yet that a certain action had been excited in the constitution. That this was the true constitutional affection of cowpox, may be judged by the acceleration of the second vesicle to a state of maturity, five days before this could have happened had there been no consentaneous general action or change in the system.<sup>6b</sup>

Two children who had been vaccinated successfully by Bryce some months before were inoculated with the virus of smallpox. In both "the variolous inoculation advanced regularly" and well formed pustules developed, in one case by the sixth day and in the other by the eighth, when they quickly dried up without "the smallest general indisposition." Here Bryce describes a clearcut example of the reaction of sensitization or partial immunity to the smallpox virus noted by Jenner and others.

Here it may be noted that in 1802 Coxe<sup>7a</sup> described accelerated and abortive reactions from vaccination and from variolation "when the system is disposed to reject" the infection (see his page 23). He gives a letter (page 70) from Benjamin Waterhouse, dated March 28, 1802, in which are described the attempts to vaccinate seven persons who had

7. Woodville, William: Reports of a Series of Inoculations for the Variolae Vaccinae, or Cow-Pox, with Remarks and Observations on This Disease, Considered as a Substitute for the Small-Pox, London, R. Phillips, 1799.

7a. Coxe, John Redman: Practical Observations on Vaccination: Or Inoculation for the Cow-pock, Philadelphia, James Humphreys, 1802.



gone through smallpox. "By the fourth day I was able to pronounce, that six of them would not have the genuine Vaccine pustule. They inflamed too quick; the inoculated part resembled the sting of a bee, and wanted the requisite hardness, as well as that deep-seated, well-defined, and slowly-progressing affection, which characterises the genuine incipient pustule." In thirteen or fourteen days scarcely any mark of the reactions was left. Waterhouse, who had been inoculated with smallpox in 1774, also describes an accelerated process from the insertion of vaccine matter in the hollow between the thumb and the finger of his left hand.

Having studied in detail several cases of second vaccination some days after the first, Bryce concludes

. . . that if, during the regular progress of cowpox, a second inoculation be performed a certain number of days after the first, the affection produced by this second inoculation will be accelerated in its progress so as to arrive at maturity, and again fade at nearly the same time as the affection arising from the first inoculation; and that this will take place although the constitutional affection be so slight as otherwise to pass unnoticed.<sup>8c</sup> . . . It is, therefore, to be wished that this test may soon be generally practised as an improvement of much importance in conducting the inoculation of cowpox, as at once giving confidence in the extent of the ailment, and precluding any necessity for any future inoculations with the virus of smallpox.<sup>8d</sup>

Briefly stated, Bryce's test was made by inoculating the other arm from the primary vesicle on the fifth day after the first inoculation. If the constitution was affected, that is, if the body was responding to the specific antigenic action of the vaccine first inserted, the vesicles on both arms would arrive at maturity together and also fade together.

## II

It is interesting to follow the reception of Bryce's proposal as reflected in the literature of the period. The test and the principle on which it is based are discussed by Bell,<sup>8</sup> Adams,<sup>9</sup> Willan,<sup>10</sup> Moore,<sup>11</sup> Monro<sup>12</sup> and others; I have not attempted to track down all the refer-

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8. Bell, George: *A Treatise on the Cow-Pox, Containing an Enumeration of the Principal Facts in the History of That Disease, the Method of Communicating the Infection by Inoculation and the Means of Distinguishing Between the Genuine and Spurious Cow-Pox*, Edinburgh, W. Laing, 1802.

9. Adams, Joseph: *A Popular View of Vaccine Inoculation, etc.*, London, R. Phillips, 1807, p. 41.

10. Willan, Robert: *Vaccine Inoculation*, London, R. Phillips, 1806.

11. Moore, James: *The History and Practice of Vaccination*, London, J. Callow, 1817.

12. Monro, Alexander (tertius): *Observations on the Different Kinds of Small Pox and Especially on That Which Sometimes Follows Vaccination, Illustrated by a Number of Cases*, Edinburgh, A. Constable & Co., 1818, p. 281.

ences to the matter. But first I must introduce the observations of Henry Hicks by way of an excerpt from Baron's "Life of Jenner."<sup>13</sup>

I have already mentioned Henry Hicks as his [Jenner's] friend and counsellor: I have also mentioned how sedulously he promoted vaccination by first submitting his own children to it, and then diffusing it in his neighbourhood; I have now to attempt to commemorate his services in another line. He made himself perfectly acquainted with all the details of cowpox inoculation; and about this time he brought this knowledge into practice. He commenced a series of inoculations; and evinced an accuracy and fidelity which would have done honour to the most enlightened physician. To show that this is no exaggerated praise I will mention that in a letter to Dr. Jenner, dated the second of August, 1799, he communicated an observation which at a subsequent period was also noticed by Mr. Bryce, of Edinburgh, and which led that gentleman to propose the only thing like an improvement in the practice of vaccination that was not suggested by Dr. Jenner himself. In two of the persons whom Mr. Hicks had vaccinated he found that "the arms came on so slowly that on the sixth day the pustules were not more forward than they generally are on the third or fourth: added to which, there appeared so much irregularity in them that I thought it better to show their arms to Mr. Darke." Two days afterwards Mr. Hicks determined to re-vaccinate them. The result he communicated in the following words: "The second inoculation seemed to make immense strides to overtake the first; and, what is wonderful, the first pustules began to change their character and put on the true vaccine appearance." He also made another remark which proves how quickly he detected every peculiarity in the progress of the affection. He adds, "I must not forget to tell you that Chamberlain's child, before the inoculation, had some eruptions on its arm and back, and as the vaccine pustule came on, these pustules assumed the exact vaccine character, and became perfect cowpox pustules." It is due to Mr. Hicks to declare that there is more original and satisfactory information to be derived from his remarks than from those of all the individuals who had written on the subject, from the time of the publication of Dr. Jenner's Inquiry.

Shortly after Bryce's book appeared, George Bell<sup>8</sup> addressed a book on cowpox to the clergy of Scotland, "who deservedly have great influence over the minds of people, and are much disposed to promote every proposal that tends to the public good." Bell discusses the work of both Woodville and Bryce. He does not approve fully of Bryce's double inoculation although he accepts the typical result as "a pretty certain test of the virus having entered the constitution." He recommends inoculation with smallpox when it is desirable to subject the patient who has passed through cowpox to the most certain test, and he describes in detail the appearance of the smallpox puncture "when the constitution is armed against the infection, by having previously passed through the vaccine disease." Three varieties of reactions are described, which obviously had been observed in the course of inoculation by Bell himself:

1. In the first of these, a few hours after the variolous matter is inserted, the puncture inflames, and continues nearly in the same state for eight, nine, or ten

13. Baron, John: The Life of Edward Jenner, London, H. Colburn, 1838.

days, the redness never varying much, or extending over a greater space than about the eighth part of an inch in diameter.

2. In the second, the puncture inflames soon after the variolous matter is inserted, and very early produces a pustule containing matter, which pustule recedes suddenly about the sixth or seventh day; and in this case may be reckoned a certain test of the patient having been previously affected with Cow-pox.

3. The last and most important variety, is that in which the pustule is inflamed on the day after inoculation, and continues to increase regularly till the tenth or eleventh day, when it is at its height, and when from the soreness of the part the patient becomes sick, and is sometimes affected with startings similar to those that precede convulsions. In this case, the inflammation is commonly of the size of a half-crown piece, but is of a darker colour than in Small-pox, and has not so much erysipelatous redness as the Cow-pox. The pustule is of a livid colour; on being punctured, it rarely gives out any matter; and where it contains a fluid, this does not readily produce any marked disease on being used in inoculation.

The inflammation begins to recede about the eleventh day; and on the fourteenth or fifteenth, is scarcely perceptible, leaving, in the place of the pustule, a brownish scab, similar to that which remains where the inoculated Small-pox has failed.

This variety, which is not uncommon, is more like the genuine Small-pox than any of the others; but it differs essentially from Small-pox in the following circumstances. It is never accompanied with any eruption in other parts of the body; the pustule does not follow the same regular progress with that of the Small-pox; and matter taken from it does not so readily propagate the Small-pox, as the virus does which is taken from a patient who is labouring under the ordinary form of the disease.

It appears, therefore, in all cases of doubt, to be a safe precaution, either to repeat the vaccine inoculation, or to inoculate the patient for the Small-pox.

Early in 1805, T. Hugo published a letter,<sup>14</sup> which I give in full:

About the sixth day, when the Vesicle on the inoculated part is formed, or about three days before the areola may be expected to come on, I insert the point of a lancet into the Vesicle, and with the lymph which exudes, I inoculate the other arm. The progress of the second Inoculation is very dissimilar to that of the first. The Vesicle proceeds more rapidly through its stages, exhibiting an areola at the same time as the Vesicle on the other arm, and being, on the ninth or tenth day, in an equal state of forwardness, but more diminutive. I consider this as so decisive a test of constitutional affection, especially in children, that unless it takes place, I never venture to warrant my patient's security against Variolous affection, but advise that the inoculation should be repeated at a subsequent period. Having acted in this cautious manner for several years, and having always taken the Virus at the earliest possible period, I have had the most satisfactory proofs of its efficacy, in resisting the contagion of the Small-pox.

In 1805, John Pearson<sup>4</sup> writes as follows:

In the early part of the year 1801, I ascertained, that if a second inoculation with vaccine fluid be performed on the sixth or seventh day after the first, a pustule will arise, which proceeds in the usual manner, until the efflorescence appears round the pustule produced by the first inoculation; and that, as soon as

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14. Hugo, T.: *M. & Phys. J.* **13**:48, 1805.

this takes place, the second pustule begins to fade, and two or three days afterwards, disappears altogether. On mentioning this as a test of the specific action of vaccine fluid on the constitution, it was suggested, that a proposal of this kind might diminish the confidence of the public in the new inoculation. I acquiesced in the objection, and did not attempt to introduce this new mode of practice.

Commenting on this statement, in 1817, James Moore<sup>11</sup> remarks:

The forbearance of Mr. Pearson at that critical period was much approved of by Dr. Jenner. . . . It is singular, that in the same year—Mr. Bryce, a respectable surgeon at Edinburgh, also made experiments similar to those made by Mr. Pearson. Each were unquestionably independent of the other; and Mr. Bryce published his in the year 1803 [1802], conceding that they would convey useful information.

This gentleman, indeed, tried the effect of revaccination during every period of the progress of a vaccine vesicle. He noticed that, when the first operation succeeded the inflammation excited by the second was accelerated; and, as soon as the primary vesicle acquired the areola, the second, however small it might be, also acquired a proportional areola, and both desiccated together.

Mr. Bryce, like Mr. Pearson, observed that this peculiarity of the second vesicle proceeded from the influence of the first; and he concluded that it might be relied upon as a sure test of the constitution being properly influenced, and secured from the Small-pox in future.

Robert Willan<sup>10</sup> in 1806 repeats that Bryce's test is founded on experiments by inoculators of smallpox, presumably Woodville in particular, which showed

. . . that if the same person was inoculated every day until the fever induced by the first inoculation supervened, all the other punctures quickly advanced in their progress, and that in the course of a day from the time the fever or general affection began, even that puncture which had been last made, perhaps only twenty-four hours before, equalled in maturity the one first made, perhaps eight or nine days before, and from which the fever had arisen.

Joseph Adams<sup>9</sup> first refers to Woodville's observation that if two inoculations of smallpox are made at an interval of three or four days, "both the inoculated places will arrive at their height at the same time." He then says: "The same has also been observed of cow-pox, and this is now so well known that the second insertion has been proposed as a test of perfect vaccination." Finally he offers the following observation as proof of the identity of the morbid poisons of smallpox and cowpox:

For if small pox is inserted to-day, and the same subject inoculated three or four days after with cow pox, or with cow pox today, and three or four days after with small pox, the same consequences will follow as if both insertions had been of small pox only, or of cow pox only; that is, each will arrive at maturity at the same time, and the only difference will be that the last insertion will produce a smaller pustule or vesicle.

Here again is a prototype of the test for antigenic immune relationship of the present day.

In 1818, Alexander Monro III writes that Bryce's test was so much approved of by Dr. Jenner that after having spent much pains in preparing a plan of his own, Jenner laid it aside after Mr. Bryce's plan was published and had the candor to state his perfect approbation of every part of it, and his utmost reliance on its efficacy in rendering the smallpox extinct.<sup>12</sup> Monro also writes:

My Father, in his Lectures, used always to express his utmost confidence in Mr. Bryce's test as a mark that the constitution has been affected, and also his opinion that its ingenious author merited a public reward; as without this improvement, he considered Dr. Jenner's discovery to be incomplete.

In reply to the objection that Bryce's test unduly complicated the process of vaccination, Monro urges, as now is the rule, that the patient should be seen more than once in order to determine whether the vaccination runs its regular course, and states that the neglect of this precaution is responsible for many of the failures.

What did Jenner think of Bryce's test for perfect vaccination? The answer in part, at least, is in the letters on the subject in Baron's "Life."<sup>13</sup>

About this time [1802] Dr. Jenner received from Mr. Bryce of Edinburgh, a copy of that gentleman's treatise on cow-pox, accompanied with a very polite letter, in which he very modestly says, that he had added only "some new hints" on the subject.

Mr. Bryce to Dr. Jenner.

Sir,

I presume to address you on the present occasion because I think it may be pleasant for you to learn that your favourite subject the inoculation of cow-pox, for which society must ever be indebted to you, has not been altogether neglected in this corner of the world.

Convinced as I am of the power of the cow-pox in shielding the human constitution from the attacks of small-pox, and being placed some time ago in a situation favourable for making observations on that interesting subject, I did not fail in marking down whatever occurrences appeared to me worthy of notice, and these I have now presumed to publish.

I have taken the liberty of desiring Messrs. Cadell and Davies, booksellers, to forward you a copy of this my publication.

You will no doubt, Sir, find a great deal of your own labours scattered throughout this essay; for your investigations on the subject have been so full and so satisfactory that it is impossible to say much on the subject without interfering with you. I trust, however, you will also find some new hints, which your ingenuity may improve farther than I have yet been able to do.

It would afford me much pleasure, Sir, if you will favour me with your sentiments on the two most prominent features of my publication; namely, the new mode proposed for obtaining and preserving the virus of cow-pox, and the proposed test of a constitutional affection. Should any objections occur to you on these points, which I have not foreseen, I trust that you will have the goodness to mention them freely; for this appears to me to be the only way by which we can arrive at truth in our investigations.

It affords me much pleasure, Sir, to observe that you are likely to reap some fruits from your labours. And while a grateful nation bestows a worldly remuneration to her benefactor, it shall be my care to . . . (the remainder of this letter is wanting).

Of the "two most prominent features," as Mr. Bryce in his essay terms them, the opinion of Dr. Jenner will be best learned from a perusal of extracts from his replies to Mr. Bryce's communications.

Dr. Jenner to Mr. Bryce, Edinburgh.

Dear Sir,

Before I enter on the subject of your present letter allow me to return you my most sincere thanks for your former one, and for your valuable publication on the cow-pox. Although you have found me so tardy in my acknowledgments, yet be assured the very handsome manner in which you mention my name in that work has not passed away without exciting in me a grateful remembrance of your own. You have anticipated some few observations that I had noted down for my next number.

You may easily conceive how incessantly I am toiling if you consider the immense extent of the correspondence that must necessarily fall upon me; and let this consideration make some allowance for my apparent neglect of your letter. I can with trust assure you that I have made efforts to answer it, fifty times since it has been in my possession.

Again, he writes thus to Mr. Bryce—April 5, 1803.

It afforded me great pleasure to find that the Vaccine virus I sent you put an end to all your solitudes respecting the perfection of that you had been previously using, and that you have diffused it so widely through the towns and villages of Scotland.

I doubt not that you will with much ease establish an institution for inoculation, on a plan equally useful with ours in London. I should send our plan, but it is not yet in print.

I much admire your precaution in using a test of the certainty of infection; and your ingenuity in the manner in which you employ it. To all young vaccinators it cannot be too strongly enjoined. The experienced will determine from the character of the pustule. The evidence before the House of Commons evinces the propriety of your observations.

I put your crust into the hands of my friend Ring, and he informed me yesterday that it had produced a good pustule. Experience now tells us that this is a good mode of sending the Vaccine virus to distant parts.

Bryce was perhaps the first to use the crust, or scab, as the source of vaccine virus. Early American writers on vaccination comment on the value of the method to them.

Bryce also describes <sup>15</sup> an immediate local redness about the point of vaccination, observed frequently in children with a skin of delicate texture, the appearances exactly resembling the inflamed spot around the sting of a bee. He regards this reaction as indicating "certain success from the operation."

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15. Bryce, James: *Practical Observations on the Inoculation of Cowpox, Pointing Out a New Mode of Obtaining and Preserving the Infection, and Also a Certain Test of Perfect Vaccination*, ed. 2, Edinburgh, W. Creech, 1809, p. 130.

In the early French <sup>16</sup> and Italian (Sacco <sup>17</sup>) experiments to determine how soon vaccination protects against smallpox, the peculiarities of the variolous eruption on inoculation soon after vaccination are described clearly. And Sacco, "the Italian Jenner," independently observed the accelerated development of the vesicles of revaccination a few days after primary vaccination. He ascribes this course to a gradual loss in disposition of the vaccinated person. I can do no better than to quote his own words in his "Trattato di vaccinazione": <sup>17a</sup>

In order to withdraw cowpox material for some time from one person, I inoculated various persons from one arm to the other, and I observed always that the last inoculation produced new pustules, which were more rapid in their course and caught up with the first pustules in the incrustation. I explain this as follows: The pustules develop in accord with the disposition of the vaccinated for smallpox, depending on the strength or weakness of which they develop more or less. Consequently the first pustules, which strike the disposition wholly uninjured, develop in full force and regularity; the disposition however can not be consumed completely at once and thus the second inoculation two or three days later will produce an effect according to the character of the remaining disposition and develop more rapidly and more irregularly. If one would inoculate again after six to seven days, it would be of no use because the disposition is then completely absent and neither a vaccine nor a smallpox pustule will appear.

The Englishman Crichton in his decision of the genuineness or spuriousness of vaccine appears to have had a similar theory in mind as he recommends to inoculate the vaccinated again after six to seven days. In case the pustules of the second inoculation by rapid and irregular course caught up with the first, then he regarded the first as regular and active; but on the other hand if the second pustules passed through a regular and undisturbed course, then the first inoculation was false and inactive. But this interpretation of vaccination would be applicable only when by some accident all the pustules were scratched off and one would be in doubt as to the preceding eruption. Otherwise the genuine cowpox is accompanied with such definite and unmistakable signs that no doubt is possible and the method consequently is superfluous and useless.

I have not succeeded in obtaining any definite inkling of the identity of the Englishman Crichton (Crichton?) to whom Sacco refers. No such name occurs in the English writings that I have read on the subject in question. Sacco's work was translated into German by Wilhelm Sprengel, <sup>17b</sup> who adds this footnote to the passage in which Sacco mentions Crichton:

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16. Rapport du Comité central de vaccine, Paris, 1803, p. 257. Bousquet, J. B.: Nouveau traité de la vaccine et des éruptions varioleuses, Paris, J. B. Baillière, 1848.

17. Sacco, Luigi: (a) Trattato di vaccinazione con osservazioni sul giavardo e vajuolo pecorino, Milan, tipog. Mussi, 1809, p. 49; (b) Ludwig Saccos Generaldirektors der Vaccination im Königreiche Italien und ersten Arztes am grossen Krankenhause zu Mailand, neue Entdeckungen über die Kuhpocken, die Mauke und die Schaafpocken (translated from the Italian by Wilhelm Sprengel), Leipzig, 1812.

Bryar [Bryce] in Edinburgh recommends to inoculate with the lymph of the first pustule 36 hours before the development of the redness about the pustule. This would be on the fifth or sixth day after the first inoculation. If now at the time the whole body is affected the progress of the second inoculation has been so accelerated that redness appears and disappears with the red zone of the first inoculation, then the inoculation has acted fully. (Edinburgh Med. and Surg. J., 1805, V. II, p. 250.) Spr

This note shows that Bryce's test was known in Germany at that time.

In 1840, Robert Ceely<sup>18</sup> described the reaction to variolous inoculation followed by vaccination in the cow in this wise: "Here was Bryce's test on the cow: Variola on one side in Vigour, tested by vaccine on the other, hastening to declare the fact of a constitutional affection."

Bryce's work did not pass unnoticed in this country. Far from it. Coxe<sup>19</sup> in Philadelphia used his method with success. Samuel Scofield,<sup>20</sup> the first resident surgeon to the New-York Institution of the Inoculation of the Cowpock, established in 1802, describes Bryce's test in detail in his book, "Vaccina or Cowpock," published in 1810. Three methods have been proposed, he says, for testing the constitutional effect of vaccination: <sup>20a</sup>

. . . the first, by Jenner, consisted in variolous inoculation soon after the person had passed through vaccina; this is a direct and clear test, but dangerous. The second method, Dr. Pearson, is a second inoculation of vaccine matter; objectionable because of practical difficulty. The third, and best, is a second inoculation during the progress of the first as proposed by Bryce.

Scofield also says "that Bryce appears to be the first who recommended the use of the scab as a substitute for the virus." Before leaving the institution named, Scofield, at the request of the medical board, tested a number of persons from among the first to have been vaccinated "by introducing the fluid matter of smallpox into both arms of each of them. On the third day from the inoculation the patients were examined, and, with one or two exceptions, the virus appeared to have taken effect; by the sixth day, however, the inflammation surrounding the punctures had entirely subsided, and not the least symptom of fever or eruption ever occurred in either of them." <sup>20b</sup> Here is described once more the specific reaction of allergy to the virus of smallpox.

18. Ceely, Robert: Observations on the Variolae Vaccinae, as They Occasionally Appear in the Vale of Aylesbury, with an Account of Some Recent Experiments in the Vaccination, Retro-Vaccination and Variolation of Cows, Tr. Provincial M. & S. A., 1840; printed in Crookshank, E. M.: History and Pathology of Vaccination, London, H. K. Lewis, 1889, vol. 2.

19. Coxe, John Redman: Observations on Vaccination, M. Repository 1:114, 1804.

20. Scofield, Samuel: A Practical Treatise on Vaccina or Cowpock, New York, Collins & Perkins, 1810; (a) p. 102; (b) p. 25.



Stephen Brown,<sup>21</sup> in his prize essay, refers to Bryce's experiments in detail, but doubts whether the test will become general on account of the additional labor and the objection of mothers. John Eberle<sup>22</sup> also refers to Bryce's test for the "sufficiency of recent vaccination," but adds that smallpox inoculation is the most certain test.

Among later writers to discuss Bryce's test, I mention especially Thomas Watson and George Gregory, whose descriptions and comments, written about 1850, merit citation. First, Gregory:<sup>23</sup>

Another variety of anomalous, or what is called modified cow pox, presents itself when the vaccine virus is reinserted on the fourth, fifth, or sixth day from the primary vaccination. The result is, that the vesicles of the secondary vaccination form rapidly, and are hurried forward in their course, so as to overtake the first crop, when the whole maturate and scab together. The secondary vesicles are thus necessarily much smaller than the primary. Mr. Bryce, in 1802, ingeniously proposed to avail himself of this law, with the view of ascertaining whether the system was under the full influence of vaccination. The plan, though it never received the sanction of Dr. Jenner [this statement is open to doubt], has nevertheless enjoyed great popularity, and is everywhere known by the name of Bryce's test. He recommends that the second application of the virus should take place on the evening of the fifth or morning of the sixth day, so that the new vesicles may have from thirty-six to forty-eight hours to grow, before constitutional or irritative fever is set up. Of late years, this procedure has fallen comparatively into disuse. In cases where the primary vaccination proves unsatisfactory, it is now more usual to recommend a repetition of the operation at the interval of one, two, or three years, according to the extent of the apparent imperfection. I believe this to be a great improvement on the plan of Mr. Bryce.

When re-vaccination is practised at distant periods from the primary insertion of the virus, the arm very generally presents the appearances of modified cow-pox—that is, the vesicles advance with abnormal rapidity. Areola forms around them on the fourth or fifth day. The resulting scabs are small, and fall off in a few days.

Thomas Watson<sup>24</sup> writes as follows:

Of course it is of much moment to determine whether the cow-pox has run its proper course or not; and it is not always easy to say how far the progress of the cow-pox may deviate from that which has just been described, without failing of its protective influence. A very ingenious test of this, free from all ambiguity, has been devised by Mr. Bryce. His plan is this. He vaccinates the other arm, or some other part of the body, four or five days after the first vaccination. If the constitution has been properly affected by the first operation, the inflammation of the second vesicle will proceed so much more rapidly than usual, that it will be at its height, and will decline and disappear, as early as that of the

21. Brown, Stephen: *Am. M. Recorder* **16**:45, 1829.

22. Eberle, John: *A Treatise on the Practice of Medicine*, ed. 2, Philadelphia, J. Grigg, 1831.

23. Gregory, George: *Lectures on the Eruptive Fevers*, Am. ed. 1 (edited by H. D. Bulkley), New York, S. S. and W. Wood, 1851, p. 253.

24. Watson, Thomas: *Lectures on the Principle and Practice of Physic*, Philadelphia, Lea & Blanchard, 1844, p. 870; 1853, p. 1157.

first: only the vesicle and its areola will be smaller. In fact, from the time of the formation of the areola, the second vesicle is an exact miniature of the first. If the system has not been duly influenced by the first vesicle, the second will run its own course, increasing up to the eighth day, and so on. Should this be the case, the second vesicle should be tested by a third.

We find the germ of this criterion in the early history of vaccination. Dr. Jenner vaccinated the children of his friend Mr. Hicks, the first gentleman who consented to adopt the practice. This Mr. Hicks became afterwards an expert vaccinator himself, and it was his custom in a doubtful case, to perform a second vaccination a few days after the first; and he remarked that the second vesicle made "immense strides to overtake the first."

Bryce's test did not survive in practice. As early as 1820 John Thomson<sup>25</sup> wrote to Bryce that "the test proposed by yourself is not generally had recourse to in this country" (Scotland). But the interest of medical writers in the significance of the changes in the reaction to vaccine and variolous virus continues to manifest itself on to the end of the century. It appears clearly, however, that the classic writings on this topic by Jenner, Bryce and others were not studied carefully. The observations and reflections of Trousseau<sup>26</sup> merit special mention. In his lectures on clinical medicine, he describes the following experiment apropos of secondary autovaccination by children:

I vaccinate: in four days I make a new puncture with a lancet charged from one of the incipient pustules; I continue to do this daily; and you have seen that up to the ninth and sometimes till the tenth day—but not later than that—there is a cow-pock developed at each new puncture. The secondary pocks, however, do not attain to the size of the primary pock, . . .

When he speaks of modified vaccinia, one is reminded of Sacco's explanation,<sup>17</sup> for Trousseau says:

. . . when the economy is in no state of aptitude for receiving or developing the virus of small-pox or cow-pox, the puncture made in vaccinating produces no more effect than if the lancet had been charged with pus from a common boil; when there is some partial aptitude, the result is abortive cow-pox at the end of some days; when there is a state of still greater aptitude, the pock, quicker in its evolution than in the normal order of events, closely resembles that of regular cow-pox; but it passes away more rapidly.

This statement anticipates clearly the interpretations today of the allergic reactions to vaccine virus as applied to problems of practical vaccination.<sup>27</sup> Trousseau's statements are reechoed in all the seven edi-

25. Thomson, John: *An Account of the Varioloid Epidemic, Which Has Lately Prevailed in Edinburgh and Other Parts of Scotland*, London, Longman, 1820; also Philadelphia, H. C. Carey, 1824.

26. Trousseau, A.: *Lectures on Clinical Medicine Delivered at the Hôtel-Dieu, Paris* (translated by John Rose Cormack), Philadelphia, Lindsay & Blakiston, 1869.

27. Leake, J. P., and Force, J. N.: *Pub. Health Rep.* **45**:2793, 1930.

tions of Flint's "Treatise on the Principles and Practice of Medicine" <sup>28</sup> published from 1863 to 1886, in this form:

During the progress of the vaccine affection vesicles having the distinctive characters of vaccinia have sometimes been observed on other parts of the body. It is probable that these are caused by the patient scratching the vesicles on the arm and carrying therefrom lymph containing the virus on the finger-nails to parts where, owing to the abrasions of the skin, self-vaccination is the result. Experiments show that between the fourth and the ninth or tenth day the characteristic vesicles may be multiplied at will by revaccinating with lymph from the vesicles produced by the primary vaccination. . . . Incomplete vaccinia—vaccinoid, as it was termed by Trousseau—is denoted by the vesicles being developed more quickly and progressing to desiccation more rapidly, by their swollen size and conoidal form, and by the absence of cicatrices or of the appearance distinctive of the true vaccine cicatrix.

In 1883, and again in 1890, Joseph Jones <sup>29</sup> in New Orleans reprinted Jenner's "Inquiry" and other early writings on vaccination, including the description from Monro of Bryce's test and Jenner's letter to Bryce, dated April 5, 1803. Jones states that in 1817 the Cow-pox Institution in Dublin reported favorably on Bryce's test, and he comments on the "simple and beautiful" demonstration of the constitutional effects of vaccination afforded by the distinctive reactions of early revaccination.

Brouardel's statement in 1898 <sup>30</sup> about the development of immunity after vaccination is remarkably lucid and comprehensive:

We have now to consider how long a time elapses after vaccination before immunity against smallpox is acquired, and how long this acquired immunity lasts. Jenner proved the reality of the immunization by submitting young James Phipps to the test of smallpox inoculation two months after having been vaccinated; it was found that he was refractory to the variolous poison. But how soon after vaccination does this immunity appear? Bryce, the Bousquet vaccine commission, Vetter, and Trousseau have determined this very exactly. They made fresh inoculations every day after the primary vaccination, and found that immunization was definite by the tenth day. Up to that time pustules developed at the seat of the puncture, but these later lesions, said Trousseau, did not reach the size of the first, those being less typical which were the result of the later vaccinations. Thus those of the ninth and tenth days aborted soon after showing a slight degree of inflammation, while after the tenth day there was no specific reaction.

Ten days after vaccination, therefore, the subject has acquired immunity to vaccina, but we have yet to see how the case stands in regard to smallpox immunity.

28. Flint, Austin: *A Treatise on the Principles and Practice of Medicine*; Designed for the Use of Practitioners and Students of Medicine, ed. 6, Philadelphia, Lea Brothers & Co., 1886.

29. Jones, Joseph: *Contagious and Infectious Diseases, Measures for Their Prevention and Arrest*, Baton Rouge, L. Jastremski, 1883. *Medical and Surgical Memoirs*; Containing Investigations on the Geographical Distribution, Causes, Nature, Relations, and Treatment of Various Diseases, 1855-1890, New Orleans, 1876-1890, vol. 3, pt. 1.

30. Brouardel, P.: *Vaccinia*, *Twentieth Cent. Pract.* 8:501, 1898.

Sacco (*Trattato di Vaccinazione*, Milan, 1809) and the committee [French] on vaccina employed the same means to determine this as were employed to determine vaccinal immunity. They inoculated children with smallpox on each day following vaccination. As long as the inoculations were not made later than the fourth day after vaccination, says Bousquet, the two eruptions developed simultaneously with the same ease and the same liberty as if each had occurred separately, preserving, however, the relations which ought necessarily to result from the difference of dates. The inoculated variola thus preserves its ordinary characters. But towards the fifth day, and especially after the sixth, while the variolous eruption occurs at the point of inoculation, there is no general eruption nor is there any febrile reaction. The inoculations made on the ninth or tenth day are followed by very poorly defined local lesions, and after the eleventh day the local symptoms fail to appear in the great majority of cases. Thus we see that smallpox immunity is acquired in almost the same time as is that against vaccinia.

Welch and Schamberg (1905)<sup>31</sup> give a clear account of Bryce's test, which they say has not been accorded much endorsement, and has fallen into disuse. Finally, it is of interest to note that the "American Illustrated Medical Dictionary"<sup>32</sup> defines Bryce's test as follows: "The determination of a degree of immunity against smallpox conferred by vaccination by repeating the inoculation after the lapse of several days: if the first is successful, the second will rapidly overtake it."

### III

Who was James Bryce?

I have been unable to learn when he was born and when he died. The few facts I can give about him and his work have been learned mostly from his publications<sup>33</sup> which are listed in the "Index Catalogue of the Library of the Surgeon-General's Office, U. S. Army."

He was fellow of the Royal Society of Edinburgh; member of the Royal College of Surgeons and president in 1816 and 1817; surgeon to the Orphan Hospital, and one of the surgeons to the Institution for the Gratuitous Inoculation of Cow-Pox, also called the Vaccine Institution of Edinburgh, founded in 1801, where he carried on the work on his test for perfect vaccination.

So far as I have been able to find, his first publication deals with an account of yellow fever in 1792 on board the *Burbridge East Indiaman*, on which he was surgeon. It was his third voyage. The ship left Downs for Madras on April 15, 1792. May 26 the equator was crossed when yellow fever broke out. Of the three hundred patients all told

31. Welch, W. M., and Schamberg, J. F.: *Acute Contagious Diseases*, Philadelphia, Lea Bros. & Co., 1905.

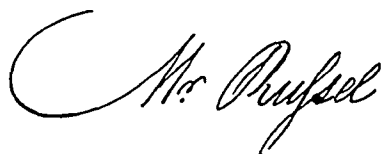
32. Dorland, W. A. N.: *The American Illustrated Medical Dictionary*, ed. 16, Philadelphia, W. B. Saunders Company, 1932.

33. There is a brief and incomplete account of Bryce in: *Biographisches Lexikon der hervorragenden Ärzte aller Zeiten und Völker*, 1929, vol. 1, p. 742. Here his name is given as James B. Bryce.

under his care, only three died. There are two copies of this account in the Army Medical Library in Washington, and both contain the handwriting of the author (fig. 3).

In addition to his book on cowpox, he wrote about variolous inoculation after vaccination in 1811, and in 1818 appeared a letter from him in the *Edinburgh Medical and Surgical Journal* on the opinions of that day in regard to the antivariolous power of vaccination.

Notes and comments on his work and reviews of his books were published from time to time in the *Annals of Medicine* and the *Edinburgh Medical and Surgical Journal* until about 1813. He wrote also (1815) on the fetal liver, and attributed the large size of the liver in the fetus at birth to the blood it contains which at birth goes into the lungs.



With respectful Compliments  
From  
The Author.

Fig. 3.—Handwriting of James Bryce.

In John Thomson's book<sup>25</sup> (Edinburgh, 1820) about a varioloid epidemic, "which has lately prevailed in Edinburgh and other parts of Scotland," are letters from Bryce in which he discusses with ability and insight the probable nature of the disease or diseases described by Thomson.

Bryce was an inventor and pioneer advocate of the stomach pump. August 4, 1824, he showed the Medico-Chirurgical Society a siphon for emptying and washing out the stomach, particularly in cases of laudanum and other forms of narcotic poisoning. At that time, and also earlier, he lived in St. Andrews Square in Edinburgh. The value of Bryce's instrument was reported promptly by Allison,<sup>34</sup> who used it with success in a case of laudanum poisoning in a man, 35 years old.

The quotations I have given from his writings show that Bryce was a keen and thoughtful observer. He recognized clearly the significance

34. Allison, P. W.: History of a Case Confirming the Efficacy of the Means of Evacuating the Stomach, in Cases of Poisoning, Proposed by Mr. Bryce, *Edinburgh M. & S. J.* **23**:416, 1824.

of the reactions on revaccination and on variolous inoculation of vaccinated persons. Whether he may be credited with absolute originality in his interpretation is of course doubtful. He himself speaks of the clue afforded him by the experiments of others with successive variolous inoculations. And it seems almost certain that he had read carefully Jenner's "Inquiry," although he makes no mention of Jenner's footnote on the change produced by smallpox or cowpox in the reactivity of the skin to variolous matter. Bryce did not hesitate to turn his observations into a practical test for perfect vaccination, hoping to increase the benefits of vaccination and to preclude the need for any further inoculations with the virus of smallpox. He promptly and properly submitted his proposed test for consideration and criticism by Jenner. Bryce thus had the power to carry into effect a measure that he believed would be of practical value.

In brief, the work by Bryce on "a certain test of perfect vaccination" is admirable. The theory was sound, the experiments adequate, the test itself simple and practical. And, devised in 1802, it has the distinction of being the first test for specific allergy.

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It was only natural that with the years the interest diminished in the peculiar reactions to smallpox and cowpox virus described so clearly and interpreted so soundly by Jenner, Bryce, Sacco and others. As smallpox yielded to vaccination, experimental human variolation ceased completely. Vaccination became a universal routine, carried out blindly, and the outcome was determined more and more "by the character of the pustule" as Jenner said would be the case. And so Bryce's test was forgotten and with it also the early records of the mysterious constitutional effects subsequently designated as allergy or sensitization. To this day all that splendid work remains neglected in the literature on immunity. The success and extent of vaccination were due in large measure to the ease with which the specific virus could be handled safely. The great principle of protective inoculation could not be applied effectively to diseases other than smallpox until Pasteur demonstrated how other viruses might be obtained in suitable forms. With the expansion of immunization, active and passive, came the discovery of serum disease and the rôle of sensitization in its manifestation and finally the complete and independent rediscovery of vaccinia allergy by von Pirquet.<sup>3</sup> If the pioneer work in that field had been known then, who would question its influence on allergic research in general or its stimulus to better understanding of the practical significance of the allergic reactions in revaccination?

# THE REACTION TO FOREIGN MATERIAL IN THE NORMAL AND IN THE INFLAMED GALLBLADDER

## AN EXPERIMENTAL STUDY

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An experimental attempt was made to determine if there exists in the wall of the normal or the inflamed gallbladder an organized group of cells or a system of histiocytes. The gallbladder is a complex histologic and cytologic structure consisting of five layers of tissue. It was considered possible that the histiocytes are arranged as a structural and functional unit of the cytologic structure of the gallbladder. The presence of an organized group of histiocytes similar to, if not identical with, the units of the reticulo-endothelial system (von Kupffer cell in the liver, reticulocytes in the spleen, and so forth) would be significant in interpreting certain cytologic, physiologic and pathologic observations.

Observations were made on dogs and rabbits. All operative procedures were made under ether anesthesia and with sterile technic. A complete necropsy was made on each animal. Sections from selected tissue were stained with hematoxylin and eosin, scarlet red, van Gieson's stain, eosin, alum-carminc or hemalum.

## PARTIAL BLOCKAGE OF THE RETICULO-ENDOTHELIAL SYSTEM WITH TRYPAN BLUE

A fine suspension of carbon material was injected intravenously into rabbits. The daily amount varied from 2 to 20 cc., and the total quantity injected varied from 0.5 to 72.8 cc. for each kilogram of body weight. At necropsy, the liver, spleen, bone marrow, lymph nodes, lungs, medullae of the adrenal glands, and the kidneys were stained black. The degree of staining varied with the quantity of carbon material that had been injected. After 30 cc. or more of carbon material for each kilogram of body weight had been injected intravenously, carbon granules could be observed in the wall of the gallbladder. The majority of carbon granules were in the cytoplasm of polyblasts and macrophages in the immediate vicinity of the capillary vessels.

Trypan blue was injected intravenously or intraperitoneally in dogs and rabbits. The wall of the gallbladder was stained blue in each case, but it was difficult to distinguish clearly extracellular or intracellular trypan blue granules.

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From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

INJECTION OF CARBON MATERIAL INTO THE LUMEN OF THE  
NORMAL GALLBLADDER

A suspension of carbon material was injected into the lumen of the gallbladder in dogs and rabbits. In no case were carbon granules observed in the wall of the gallbladder or in the regional lymph nodes.

INJECTION OF CARBON MATERIAL INTO THE WALL OF THE  
NORMAL GALLBLADDER

In several dogs, a fine suspension of carbon material was injected into the wall of the gallbladder. The animals were killed from four to eight days later. The carbon material was entirely phagocytosed by polyblasts and macrophages of local origin. Phagocytic cells were in every layer of the gallbladder, but were most numerous in the tunica fibrosa externa.

INJECTION OF VARIOUS TYPES OF FOREIGN MATERIAL INTO THE  
WALL OF THE INFLAMED GALLBLADDER

In several dogs, acute hemorrhagic cholecystitis was produced by the intravenous injection of Dakin's solution. Carbon material or cholesterol with emulsified fat was then injected into the wall of the gallbladder. The animals were killed from two to four days later. The carbon material and globules of fat were observed in the cytoplasm of numerous polyblasts and macrophages. Only a few particles of the injected material were extracellular. It appeared as if the inflammatory reaction produced increased phagocytic activity.

## RESULTS

When vital dyes or suspensions of finely particulate material are injected intravenously, they are quickly removed from circulation. The cells responsible for this elimination are the units of the reticulo-endothelial system, which are principally situated in the sinusoids of the liver, spleen, bone marrow, lymph nodes and medulla of the adrenal gland. Small quantities of graphite injected intravenously into rabbits disappeared rapidly from the peripheral blood stream. At necropsy, the organs mentioned as containing special divisions of the reticulo-endothelial system were uniformly colored black. Particulate carbon was never demonstrated in the wall of the gallbladder of the rabbit following the intravenous injection of small quantities of graphite. Only after the more advantageously situated reticular cells, such as the phagocytic endothelial cells lining the sinuses or sinusoids of the liver and spleen, had been partially saturated, was any of the graphite deposited in the wall of the gallbladder. The quantity of particulate material necessary to produce this primary saturation varied somewhat with the state of the animal, but seemed to occur quite uniformly after the injection of 30 cc.



of graphite preparation for each kilogram of body weight. This condition may be compared to a physiologic threshold of the reticulo-endothelial system for particulate matter.

The first carbon particles that appeared in the wall of the gallbladder following intravenous injection were in the cytoplasm of the cells immediately surrounding the smaller capillaries. They increased in size and number as the total quantity of carbon injected into the animal was increased. At no time could endothelial cells be observed to detach themselves from the wall of the vessel, nor could they be found in the wall of the gallbladder as single carbon-containing units. This is in agreement with Lang's<sup>1</sup> observation on the endothelium of the capillaries in aseptic inflammation. Many peculiar cells were concentrically arranged about the peripheral portions of the endothelial cells. These cells, which have been designated as pericytes by Zimmermann,<sup>2</sup> were not only arranged about the arterioles, capillaries and the larger vessels, but appeared as detached individual cells, free in the tissues. In the transition from a fixed to a detached, mobile state, they changed from a thin, curved cell, with a central, oval nucleus, to a round or oval cell, similar in appearance to a small polyblast. In many sections, cells about the vessels displayed such remarkable resemblance to lymphocytes that a hematogenous origin had to be considered as a possibility. At no time, however, were any lymphocytes observed in migration from the lumina of the vessels to the tissue. Granular leukocytes were not found in the wall of the gallbladder. The entire process presented the appearance of a mild, localized, cellular reaction to an aseptic inflammation. It appears, therefore, from the data at hand, that most if not all of the phagocytic cells were derived from resting wandering cells, polyblasts, pericytes and histiocytes.

Particulate carbon produced intense irritation when injected directly into the wall of the gallbladder. The type and uniformity of the cellular response were remarkable. The cells that contained the particulate carbon were arranged in three superimposed, concentric layers. The first layer was in the submucosa, the second layer in the large connective tissue septums of the muscularis, and the third layer in the tunica fibrosa externa. These have been called the internal, middle and peripheral layers. The peripheral layer was the thickest. In these laminae the majority of cells were round, oval or polygonal, and their size varied from about 15 to 40 microns in diameter. In observing the structure of the graphite-containing cells it is essential to realize that the carbon is frequently crowded into every available portion of the cytoplasm.

The reaction to foreign material in the wall of the experimentally inflamed gallbladder consisted of a combination of the cell types observed

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1. Lang, F. J.: *Arch. Path.* **1**:41, 1926.

2. Zimmermann, K. W.: *Ztschr. f. Anat. u. Entwicklsgesch.* **68**:29, 1923.

when the two conditions were produced separately. When particulate carbon and cholesterol had been injected into the wall of the gallbladder, it was possible to distinguish granules of carbon material and globules of cholesterol in the cytoplasm of many histiocytes. The phagocytosis of fat by the histiocytes of the subcutaneous tissue of rabbits has been described by Kusnetzowsky<sup>3</sup> and Anitschkow.<sup>4</sup> The cells that phagocytose fat in the subcutaneous tissue of the rabbit are apparently morphologically similar to the histiocytes that phagocytose foreign material introduced into the wall of the gallbladder of the dog.

The histiocytes of the gallbladder of man may be instrumental, if not a decisive factor, in restricting many inflammatory processes. The gallbladder is exposed to infection from two surfaces. The mucosa is in direct contact with the contents of the lumen, which may and frequently does contain virile, pathogenic organisms. Cholecystitis is frequently restricted to the mucosa and submucosa. In pericholecystitis, however, the inflammatory changes are restricted to the outer layers. The active participation of the histiocytes of the gallbladder in the defense against bacterial invasion may be a larger factor in arresting or limiting injury.

#### SUMMARY

The reaction to foreign material in the wall of the normal and the inflamed gallbladder was observed. It was found that in the gallbladder of the dog and rabbit the histiocytes, owing to the distribution of the connective tissue, are arranged in three concentric layers. The majority of the histiocytes in the wall of the gallbladder, which under normal conditions are in an inactive stage, appear to develop from a preexisting local cell. The injection of particulate matter or vital dyes directly into the wall of the gallbladder of the dog produces rapid mobilization of the resting wandering cells into large, active, mobile, phagocytic histiocytes. These cells are identical with the histiocytes found in the connective tissue throughout the body. There is a possibility that a few histiocytes may develop from mononuclear, nongranular, hematogenous leukocytes, which have become lodged in the wall of the gallbladder. This must be considered as an accessory source. Histiocytes were demonstrated in the wall of the gallbladder of the rabbit by the use of vital stains. Carbon material was not absorbed from the lumen of the gallbladder of the dog or rabbit. In chemical cholecystitis, the inactive histiocytes are mobilized in large numbers. They rapidly phagocytose particulate matter, cholesterol and olive oil injected into the wall of the gallbladder. The arrangement of the histiocytes in three concentric layers suggests their function as a phagocytic cellular defensive mechanism in cholecystitis and other pathologic conditions of the gallbladder.

3. Kusnetzowsky, N.: *Arch. f. mikr. Anat.* **97**:32, 1923.

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# PRODUCTION OF GASTRIC AND DUODENAL ULCERS IN EXPERIMENTAL CINCHOPHEN POISONING OF DOGS

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AND

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These experiments were begun in an effort to produce in dogs the 'acute degenerative changes in the liver alleged to occur occasionally in human patients who have taken cinchophen. The effect of cinchophen on the canine liver has been reported previously.<sup>1</sup> Postmortem examinations revealed that a large percentage of these animals had gastric and duodenal ulcers. This paper is a report of this phase of these experiments, which indicate that the feeding of cinchophen is the most effective means yet introduced for producing gastric and duodenal ulcers in dogs without operative procedures that disturb the normal anatomic relations of stomach and intestines.

## PERTINENT FACTS FROM THE LITERATURE

Experimental work on the etiology of peptic ulcer began late in the nineteenth century with the work of Cohnheim. The abundant literature which has developed since that time has been so frequently reviewed that only the briefest reference to it is necessary in this paper. It is now known that the factors which may induce acute gastric erosions are not necessarily the same as those which produce the typical chronic indurated ulcers, although it seems probable that all chronic ulcers originate on the basis of acute erosions. Much confusion has arisen from the fact that many writers have failed to differentiate between factors tending to produce acute erosions and those tending to induce chronicity.

*Etiologic Factors in Acute Erosions.*—The numerous suggested factors in the causation of acute gastric erosions may be classified as circulatory, physical, chemical, bacteriologic and miscellaneous.

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Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Churchill, T. P., and Van Wagoner, F. H.: Proc. Soc. Exper. Biol. & Med. 28:581, 1931.

Virchow believed that infarction following embolism was the basis of the formation of gastric ulcers. Cohnheim<sup>2</sup> and others produced ulcers by injecting suspensions of metallic salts. Ivy<sup>3</sup> repeated this work, but failed to produce lesions when using an inert material. Von Bergmann<sup>4</sup> and Westphal<sup>5</sup> believed that arterial spasm was the cause of obstructed circulation.

The physical agents that have produced ulcers of the stomach are: x-rays (Wolfer<sup>6</sup>), heat (Decker<sup>7</sup> and Katzenstein<sup>8</sup>) and intense cold (Ribbert<sup>9</sup>). Excision of portions of the mucosa and of the mucosa and muscularis have been tried, but these defects heal with remarkable rapidity unless other factors are present, such as unabsorbed suture material.

The chemical agents may be subdivided into local escharotics and chemical and biologic poisons. Acute erosions have been produced by submucous injections of various chemicals, the most commonly used being silver nitrate. Superficial burns are sometimes associated with acute gastric erosions. This has been explained on a toxic basis. Gastrototoxic serums that cause ulcers have been produced experimentally. Ivy and Shapiro<sup>10</sup> produced gastric ulcers by means of local anaphylaxis.

Bacteria as possible factors in the production of ulcers have been advocated chiefly by Rosenow,<sup>11</sup> who reported the isolation of a specific strain of streptococci.

Berg and Jobling<sup>12</sup> believe that there may be a biliary or hepatic factor in the formation of some peptic ulcers. They observed gastric or duodenal ulcers in about 50 per cent of dogs with a Peyton-Rous permanent biliary fistula. Other etiologic factors that have been advocated are: a preexisting gastritis, embryonal rests in the gastric mucosa, vitamin deficiencies and endocrine disturbances.

*Factors Inducing Chronic Changes in Acute Lesions.*—Anatomic, mechanical and chemical factors are foremost in the formation of chronic peptic ulcer. Aschoff<sup>13</sup> believed that lesions along the lesser

2. Cohnheim, J.: Lectures on General Pathology, London, the New Sydenham Society, 1890, vol. 3, p. 875.

3. Ivy, A. C.: Arch. Int. Med. **25**:6, 1920.

4. von Bergmann, G.: Jahresk. f. ärztl. Fortbild. **12**:3, 1921.

5. Westphal, K.: Deutsches Arch. f. klin. Med. **114**:325, 1914.

6. Wolfer, J. A.: J. A. M. A. **87**:725, 1926.

7. Decker: Berl. klin. Wehnschr. **24**:369, 1887.

8. Katzenstein, M.: Berl. klin. Wehnschr. **45**:1749, 1908.

9. Ribbert, H.: Frankfurt. Ztschr. f. Path. **16**:343, 1915.

10. Ivy, A. C., and Shapiro, P. F.: Arch. Int. Med. **38**:237, 1926.

11. Rosenow, E. C.: J. A. M. A. **61**:1947, 1913.

12. Berg, B. N., and Jobling, J. W.: Arch. Surg. **20**:997, 1930.

13. Aschoff, Ludwig: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 279.

curvature and prepyloric region of the stomach tend to become chronic because of the peculiar anatomic arrangement of this portion of the stomach, which he called the "Magenstrasse." In this region, the mucosa is arranged in taut longitudinal folds, and the blood vessels to this region are more indirect than elsewhere, and the circulation is interfered with by the muscular contractions of the stomach. The experimental work of Ivy,<sup>14</sup> Mann and Williamson<sup>15</sup> and McCann<sup>16</sup> lends support to the theory that mechanical factors play a part in the formation of chronic peptic ulcers. Surgical procedures have hitherto offered the best method of studying the effect of the acid gastric juice on the formation and healing of ulcers. Mann and Williamson<sup>15</sup> and others devised operations for shunting the alkaline content of the duodenum into some portion of the intestine other than the normal. By this method the gastric juice was thrown into an intestine deprived of the neutralizing secretions. These procedures produced acute and chronic ulcers in the intestine in a large percentage of the experiments. In addition to the factors named, there is indication that such factors as nutritional disturbances, toxemia and metabolic disturbances may play a part in the chronicity of ulcerative gastric lesions.

#### CINCHOPHEN

Cinchophen, or phenylquinoline carboxylic acid, was introduced into medicine in 1908 by Nicolaier and Dohrn. Since that time its use has increased in popularity until it is now employed extensively by physicians in the treatment of rheumatism, arthritis and gout. It is also widely used by the laity. In 1923, Worster-Drought<sup>17</sup> described the first case of toxic manifestations presumably due to cinchophen. Since that time eighty-one clinical cases of alleged cinchophen poisoning have been reported. In the cases in which autopsy was performed, the essential pathologic change consisted of acute yellow atrophy of the liver, and in only three of the few reports in which the gastro-intestinal tract is mentioned, are the gastro-intestinal lesions described: gastritis, petechiae of the intestinal mucosa and aphthous ulcers of the mouth.

#### METHODS

Well nourished, apparently healthy dogs were used in the experiments. Each dog was given a daily dose of cinchophen, which had been thoroughly mixed with a meat and cereal preparation. They received the usual care accorded laboratory animals. In the majority of cases, the dogs were allowed to eat or not as they chose, and no forced feedings were employed. The rest of the dogs were fed in the same manner until food was persistently refused; then a varying number of

14. Ivy, A. C.: J. A. M. A. **75**:1540, 1920; **85**:877, 1925.

15. Mann, F. C., and Williamson, C. S.: Ann. Surg. **77**:409, 1922.

16. McCann, J. C.: Arch. Surg. **19**:600, 1929.

17. Worster-Drought, C. C.: Brit M J. **1**:148, 1923.

doses of cinchophen, suspended in cottonseed oil, were given by stomach tube. The daily dose of the drug administered was calculated on the basis of the average human therapeutic dose. If 150 pounds (68 Kg.) is considered as the average weight of the human body and 7.5 grains (0.49 Gm.) three times a day as the average dose, the human dose is approximately 0.33 grains (0.022 Gm.) per kilogram of body weight. The dogs were weighed at the beginning of the experiment, and the dose was calculated on the basis of this initial weight.

The dogs were closely observed as to symptoms, and postmortem examinations were made as soon after death as possible. A few, when their condition became extremely bad, were put to death under ether anesthesia. The stomachs were immediately removed, opened along the greater curvature and washed out. Some of the specimens were retained as gross specimens, but the majority were sectioned and stained by the routine hematoxylin and eosin method for microscopic examination. Portions of several of the ulcer-bearing stomachs were fixed in absolute alcohol and stained for mucin content by the mucicarmine method.

The lesions classified as acute ulcers were carefully differentiated from areas of postmortem digestion by microscopic demonstration of an inflammatory reaction in addition to the break in the mucosal continuity. Lesions were considered chronic only when deep penetration and reactive fibrosis could be demonstrated. Healing was determined by scar formation and contraction of the mucosa.

#### OBSERVATIONS

*Number of Ulcers Produced.*—The dogs were divided into four groups, depending on the daily dosage of cinchophen.

Group 1, consisting of five dogs, received twenty-seven times the normal human dose per kilogram (i. e., twenty-seven times 0.022 Gm. per kilogram). Two received 10 doses, and the rest 9, 8 and 3 doses, respectively. In the first three of these dogs ulcers developed.

Group 2 received ten times the normal dose per kilogram. The seven dogs in this group receiving 123, 24, 22, 20, 10, 8 and 8 doses, respectively. Ulcers developed in every dog.

Group 3 received five times the normal human dose per kilogram. There were five dogs in this group, receiving 118, 58, 21, 15 and 13 doses, respectively, and ulcers developed in all.

Group 4 received the normal human dose per kilogram. The seven dogs in this group received 101, 79, 71, 56, 6, 3 and less than 10 doses, respectively. Ulcers developed in the first four.

The results, as summarized in table 1, show that of the entire series of twenty-four dogs, nineteen, or approximately 80 per cent, acquired true gastric ulcers.

The total amount of cinchophen per kilogram of body weight necessary to produce gastric ulcers varied considerably. Thus, dog 20 showed ulcer formation after ingestion of only 1.23 Gm. of cinchophen per kilogram, while dog 15 was given 27.06 Gm. per kilogram. The average total amount ingested per kilogram was 5.06 Gm.

The minimum time required for the production of the ulcers cannot be stated with certainty, since there was no way of determining how

long the lesion had been present when autopsy was performed. However, dog 33 died from intercurrent infection eight days after the beginning of the experiment and showed acute ulcer formation. Likewise, dog 1 died from perforation of an ulcer after ingesting daily doses of the drug for a period of ten days.

*Symptoms.*—All of the dogs, except dog 1, early presented anorexia, which persisted throughout the course of the experiment. The majority of the dogs, in addition, showed occasional nausea and vomiting. Those which developed ulcers had tarry stools at intervals.

TABLE 1.—*Summary of Experimental Data*

Group	Dog	Initial Weight, Kg.	Total Amount Drug per Kg. Body Weight, Gm.	Total Dose, Gm.	Number of Doses	Length of Experiment, Days	Result
1 27 × N.H.D.* 0.6 Gm. per Kg.	1	6.3	6.0	37.8	10	10	Ulcer
	2	7.8	6.0	46.8	10	20	Ulcer
	4	7.8	5.4	42.1	9	15	Ulcer
	5	20.3	4.8	97.4	8	55	Negative
	6	23.5	1.8	42.3	3	20	Negative
2 10 × N.H.D. 0.22 Gm. per Kg.	15	6.1	27.06	165.1	123	156	Ulcer
	19	11.2	5.74	64.3	24	51	Ulcer
	34	8.3	4.84	40.2	22	25	Ulcer
	12	17.2	3.87	66.6	20	20	Ulcer
	25	8.6	2.00	17.3	30	30	Ulcer
	11	10.5	1.76	18.5	10	10	Ulcer
	33	8.3	1.76	14.6	8	8	Ulcer
3 5 × N.H.D. 0.11 Gm. per Kg.	22	16.2	12.95	209.8	118	143	Ulcer
	18	11.9	6.56	77.8	58	79	Ulcer
	24	8.7	2.36	20.5	21	47	Ulcer
	16	8.9	1.68	14.9	15	21	Ulcer
	23	12.4	1.42	17.5	13	26	Ulcer
4 N.H.D. 0.022 Gm. per Kg.	17	13.6	2.26	30.6	101	121	Ulcer
	8	12.3	1.75	21.5	79	96	Ulcer
	13	9.2	1.56	14.3	71	77	Ulcer
	20	12.5	1.23	15.4	56	113	Ulcer
	9	8.7	0.13	1.1	6	7	Negative
	21	16.2	0.06	1.0	3	6	Negative
	7	9.3	?	?	10	137	Negative
Average.....			5.06	(or less)			
Ulcers produced in 19 of 24 dogs, or 80 per cent							

\* N.H.D. = normal human dose.

There was a marked loss in weight in all the animals (table 2). This loss varied from 0.3 to 7.5 Kg., with an average loss in weight of approximately 3 Kg. Some of the animals lost as much as 44 per cent of their body weight during the course of the experiment, and—taking the group as a whole—there was an average weight loss of 23 per cent. This average loss per day was about 0.7 per cent.

*Distribution of Ulcers.*—The distribution of the ulcers was characteristic. In four of the dogs multiple gastric erosions were distributed widely throughout the mucosa. One dog showed only a single erosion, which was near the pylorus. There were thirty-four chronic gastric ulcers produced in these twenty-four dogs, and approximately 90 per

cent of them were located in the gastric pathway, including the entire pyloric canal, and about 70 per cent of the ulcers were found on the lesser curvature. The duodenal ulcers were all in the first portion of the duodenum, three being located on the anterior wall and one on the posterior wall.

*Morbid Anatomy.*—On pathologic examination, the lesions found in the stomach and duodenum were morphologically identical with the classic descriptions of gastric erosions and peptic ulcers. The following protocols indicate the types of lesions found.

DOG 1.—The peritoneal cavity was filled with a brownish, foul-smelling fluid, and the serous coverings showed marked hyperemia and were covered with fibrino-

TABLE 2.—*Summary of Effects of Cinchophen Poisoning on Weight of Animals*

Dog	Initial Weight, Kg.	Weight at Death, Kg.	Length of Experiment, Days	Weight Loss, Kg.	Weight Loss, per Cent	Weight Loss per Day, per Cent
1	6.3	6.0	10	0.3	4.7	0.47
2	7.8	6.4	20	1.4	17.9	0.90
4	7.8	6.6	15	1.2	15.4	1.03
5*	20.3	12.2	55	6.1	30.0	0.55
6*	14.1	11.3	20	2.8	20.0	1.00
7*	9.3	6.2	137	3.1	33.3	0.24
8	12.3	8.1	96	4.2	34.1	0.36
9*	8.7	8.0	7	0.7	8.1	1.16
11	10.5	9.2	10	0.7	6.7	0.66
12	17.2	11.5	20	5.7	33.1	1.65
13	9.2	5.7	77	3.5	38.0	0.49
15	6.1	4.1	156	2.0	32.8	0.21
16	8.9	7.1	21	1.8	20.2	0.96
17	13.6	9.5	121	4.2	30.6	0.25
18	11.9	7.8	70	4.1	34.4	0.44
19	11.2	8.2	51	3.0	26.8	0.53
20	12.5	7.0	113	5.5	44.0	0.39
21*	16.2	14.8	6	1.4	8.6	1.43
22	16.2	8.7	143	7.5	46.3	0.32
23	12.4	8.4	26	4.0	32.2	1.24
24	8.7	6.0	47	2.7	31.0	0.66
25	8.7	6.7	30	2.0	23.0	0.77
33	8.3	8.0	8	0.3	3.3	0.41
34	8.3	6.0	25	1.4	16.8	0.67
Averages.....				2.89	23.0	0.70

\* Indicates dogs in which no ulcers developed.

purulent exudate. On the lesser curvature of the stomach, 1 cm. proximal to the pylorus, there was a small opening in the serosa, through which gastric contents could be expressed. When the stomach was opened, an ulcer was found opposite the perforation in the gastric wall. There was a moderate degree of hyperemia of the duodenum. This specimen was mounted, and no sections were made.

DOG 4.—The peritoneum was normal, except for a small, roughened, dull and lusterless area surrounded by a zone of hyperemia on the posterior wall of the stomach. When the stomach was opened, three ulcerated areas were found. These varied from 4 to 8 mm. in diameter. Two of these ulcerations were located in the pyloric canal, one on the lesser curvature and one on the posterior wall. The remaining ulcer was located on the anterior wall of the stomach just proximal to the isthmus. This ulcer microscopically showed destruction of the mucous membrane, the submucosa and portions of the muscular coat and was surrounded by an acute inflammatory reaction with very little fibrosis. The two pyloric ulcers showed deep penetration into the muscular coat. Microscopic section of one of



these ulcers showed absence of the mucosa, submucosa and a large part of the muscularis; its floor was covered with fibrin, which contained a few polymorphonuclear leukocytes; beneath this area were many polymorphonuclear leukocytes and red blood cells, and a large amount of fibrin. Still deeper was a zone of typical granulation tissue with a number of lymphocytes. Surrounding the ulcerated area there was a marked increase of fibrous tissue in the submucosa, muscular coat and serosa. This fibrous tissue contained distended blood vessels and polymorphonuclear leukocytes. The serosal surface was covered with fibrin and polymorphonuclear leukocytes. The marginal epithelium showed a slight downgrowth along the margins of the ulcer.



Fig. 1 (dog 19).—Early formation of ulcer.

Dog 19.—The stomach contained a small indurated ulcer on the lesser curvature involving the pyloric ring. The mucosa around this area was drawn together in purse-string fashion. A similar ulcer was located on the posterior wall about 2 cm. from the pyloric ring. Multiple superficial erosions were also present. Microscopic section of one of these erosions showed destruction of the mucous membrane and some involvement of the submucosa. The muscularis was not involved. The entire area was surrounded with polymorphonuclear leukocytes and lymphocytes, but showed no fibrosis. Microscopic sections from this stomach showed what is believed to be one of the earliest stages of gastric ulcer. One long crypt extended to the muscularis and ended in a flattened dilatation. The columnar epithelial cells covering the part resting against the muscularis were necrotic and covered with a layer of fibrin and polymorphonuclear leukocytes. Radiating from this area into the underlying tissue there were numerous polymorphonuclear leukocytes and a few large cells with a clear cytoplasm and a single vesicular nucleus. The vessels in this area were distended with blood (fig. 1).

The first portion of the duodenum showed an ulcerated area on the posterior wall measuring 6 by 12 mm. This ulcer was firm, had a necrotic base and on section showed some degree of fibrosis associated with a zone of inflammation. On the lesser curvature of the stomach, slightly posterior and adjacent to the pylorus, was a large indurated ulcer, 2 by 4 cm. in size, which had penetrated almost completely through the wall of the stomach. It had a necrotic base; its proximal and lateral borders were fairly smooth and undermined, and the distal border showed the familiar terraced appearance.

Mucicarmine stains on all sections showed a relative deficiency of mucin compared with sections taken from the same relative locations in the stomachs of normal dogs.



Fig. 2 (dog 23).—Chronic gastric ulcer with perforation.

Dog 23.—In the lesser peritoneal cavity there was a small circumscribed area of inflammation of the serosal coat of the stomach which was bound down by adhesions to the adjacent peritoneal surfaces. On tearing these adhesions, a quantity of foul-smelling fluid escaped from the stomach. In the stomach opposite this there was a large ulcer, 2 by 4 cm. in size, which had eroded completely through the wall of the stomach, but perforation had been prevented by protective adhesions. The ulcer showed overhanging edges, especially in the distal portion. Numerous eroded thrombosed blood vessels were visible in the base of the ulcer. In the fundus and along the lesser curvature there were several superficial erosions. The duodenum contained one slightly indurated ulcer on the posterior wall, proximal to the ampulla of Vater (fig. 2).

#### COMMENT

From the foregoing results it is apparent that cinchophen exerts a markedly deleterious influence on the gastro-intestinal tract of the dog.

This may be a species-selective action, but the reported clinical cases of cinchophen poisoning show symptoms referable to the gastro-intestinal tract. Schroeder described pyrosis, nausea, vomiting, diarrhea and anorexia in patients taking cinchophen. Apparently, then, the drug does not produce gastric disturbance in dogs only. Unfortunately, interest in the human cases reported has been centered on the liver, and little has been said of the gastro-intestinal tract.

*Advantages of Method for Production of Ulcers.*—Apart from the possible clinical application, the production of gastric and duodenal ulcers by the administration of cinchophen makes available a new approach to the experimental study of the formation of ulcers.

Eighty per cent of this series of twenty-four dogs acquired gastric ulcers, a lesion that occurs spontaneously with extreme rarity and has been produced experimentally with considerable difficulty in dogs. This number is as high as, or higher than, the number of ulcers produced by any other method hitherto reported. The five dogs that showed no ulcers ate the drug at infrequent intervals over a long period of time. Dog 5 had 8 doses over a two months' period. Dog 6 had 3 doses in twenty days. Dogs 9 and 24 had only 6 and 3 doses, respectively, and although the drug was administered regularly the dose and the total amount given were very small. Dog 7 had 10 doses in one hundred and thirty-seven days. It is apparent, then, that cinchophen, if given regularly over a sufficient period of time, will produce ulcers in almost 100 per cent of dogs.

From the reports in the literature the production of chronic gastric ulcer has been difficult, and the results have been variable. The one exception to this is the prolonged use of the roentgen ray as reported by Wolfer. Intestinal ulcers have been produced in a large percentage of dogs by use of the Mann-Williamson duodenal drainage operation or its modifications. These operative procedures disturb the normal anatomic relationship of the gastro-intestinal tract and consequently the physiologic relationship. Chronic gastric and duodenal ulcers in animals have not hitherto been produced by any of the mechanisms that operate in the formation of human peptic ulcers. By cinchophen feeding the normal anatomic arrangement is not disturbed, and thus the confusing factors that may result from trauma and from the malposition of these organs are eliminated.

*Theories as to Pathogenesis.*—The mechanism by which these ulcers are produced by cinchophen has not been determined. The possibilities suggested by these experiments are: 1. Cinchophen may have a direct toxic action on the gastric mucosa, combined with digestion by gastric secretions. 2. The drug may combine with the mucin of the stomach and thus remove the normal protection of the gastric mucosa. 3. It

may act on the autonomic nervous system and induce erosions by its neurogenic effect. 4. Cinchophen may influence the secretion of gastric juice, either directly or through the endocrine system. 5. The drug may have a general toxic action which affects the stomach directly or by causing metabolic disturbances as a result of its effect on the liver or pancreas. 6. The nutritional disturbances resulting from the anorexia that follows the ingestion of cinchophen may be a factor in the production of the ulcers.

Work is now in progress to determine, if possible, which of these factors is responsible for the production of gastric and duodenal ulcers in dogs fed with cinchophen.

#### SUMMARY

Acute and chronic gastric ulcers were produced in nineteen of twenty-four dogs. In four of these dogs ulcers were also found in the duodenum.

The five dogs in this series in which gastric ulcers did not develop were either fed inadequate doses or administered doses were distributed irregularly over such a long period of time that this effect of the drug was lost. Apparently, then, if cinchophen is given regularly to dogs over a sufficient length of time, gastric ulcers will result in practically all the animals.

The dose of cinchophen producing ulcers in all dogs is from 0.11 to 0.22 Gm. per kilogram per day.

The acute gastric ulcers were distributed widely over the gastric mucosa; 90 per cent of the chronic gastric ulcers were along the gastric pathway, or "Magenstrasse."

The duodenal ulcers were located above the ampulla of Vater and, in one case, occurred on both the anterior and the posterior wall.

The acute and chronic ulcers of both the stomach and the duodenum were identical with human peptic ulcers in their gross morbid anatomy and histology.

# Laboratory Methods and Technical Notes

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## TWO-COLOR PHOTOMICROGRAPHIC LANTERN SLIDES FROM ORDINARY MATERIALS

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The usefulness of photomicrographic lantern slides in color for the demonstration of histologic details is universally conceded, but the technical difficulties in the production and projection of such slides deter many from their use. Of all the appliances used in color reproduction, none equals the screen plate in convenience or excels it in the faithfulness with which colors are recorded. For viewing as transparencies or for color reproduction, plates of this type are of the greatest value. Very satisfactory lantern slides may be made also by their use, but difficulties arise in their projection, since the color screen absorbs from 85 to 90 per cent of the light. In order to form a sufficiently brilliant image on the projection screen, it is necessary that a source of intense light be employed. Hence, these plates are poorly suited for projection in company with ordinary black and white plates, since an intensity of light that is barely satisfactory for the screen plates gives a painfully bright image with the usual monochrome slide.

Color separation methods in which separate color positives are superimposed for projection have long been known, and such procedures form the basis of color process commercial printing and color cinematography.

Full color reproduction requires the use of three color images. However, if the specimen to be photographed can be rendered in two colors, the use of tricolor methods is obviously extravagant. The majority of the sections of tissues used by the pathologist are stained with red or blue dyes, or with combinations of these two colors. Thus complete color reproduction can be obtained by two-color methods, at once greatly simplifying the process. By properly standardizing the various procedures, it is possible to produce satisfactory lantern slides of such transparency that they may be intermingled with ordinary black and white slides without visual strain. Since the positives are prepared from a pair of negatives, an indefinite number of duplicates may be made. In addition, one of these negatives may be used for black and white prints.

### METHOD

*Illumination.*—The carbon arc and the incandescent lamp are the chief sources of illumination used in photomicrographic work at the present time. Various types of automatic carbon-feeding devices are available and, with many of them, a fairly uniform arc can be obtained.

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Of the incandescent lamps available, the 6 volt ribbon filament lamp is probably the most satisfactory. If the light is used from a small portion of the filament, it is very uniform, while the intensity is adequate for any photographic purpose, provided that the condenser system is carefully set up.

The light for color work must be constant in intensity and composition. In these respects, the incandescent lamp is superior to the carbon arc light and also is far more convenient. For the most critical work, the lamp may be provided with an ammeter and rheostat, so that the operating conditions may be consistently reproduced. Where the voltage supplied is reasonably constant, such additional equipment seems to be an unnecessary refinement.

*Optical System.*—The entire optical system from the condenser to the ocular should be of the apochromatic type. It must be emphasized that color work with any but the most highly color-corrected lens systems available is a waste of time. With achromatic lenses which are satisfactory for visual work, the separate color images will not be formed in the same plane and of the same size, so that registration is impossible. Special attention should be given the ocular to insure that it is adapted to the particular objective used; otherwise the fine color corrections of both lenses may be impaired.

*Photographic Material.*—For several reasons, glass plates will be found more satisfactory for negatives than cut films, although the latter are more convenient to store. The rigidity of the base makes the glass plate free from distortion, and it may also be more conveniently handled in the subsequent placement of registration marks and in printing.

Panchromatic emulsions are a necessity. Of the many excellent plates available, the Wratten and Wainwright "M" plates are probably the most satisfactory. These are moderately rapid plates with an unusually fine grain and give practically any desired degree of contrast.

The plate should be 4 by 5 inches (10.16 by 12.7 cm.). This gives a generous margin about the lantern slide positive in printing, so that a slight movement of the camera back in changing plate-holders at the time of the exposure is of no importance. Since the prints are made by contact, any larger plate results in a waste of material.

For the positives, the Eastman slow lantern slide plates are used.

*Exposure.*—The plate-holders are loaded in absolute darkness, and one holder should have some marking device to cast a shadow on the corner of its plate. This may be a small metal strip tacked to the holder or a piece of paper pasted to the kit holding the plate. If the marked holder of each pair is always used with the same filter, the negatives can thus be easily distinguished.

The photomicrographic equipment having been set up in the usual way, a sharp image is focused on the ground glass in the size desired for the lantern slide. The image must be uniformly sharp and absolutely free from color fringes. When the image is satisfactory, the exposures are begun and no more adjustments of the camera or of the microscope are attempted. The shutter is closed, and a holder carrying a Wratten "F" filter is placed on the optical bench. This is a deep red filter, passing less orange than the usual tricolor red filter. A loaded plate-holder is inserted, the slide drawn and the exposure made. The plate-holder is removed, and without disturbing the camera in any way, the second holder is inserted, and the red filter is replaced by a combination of the Wratten "B" and "H" filters. These filters together give a pure green and are practically opaque to red light. The second exposure is then made by green light, and the plates are ready for development.

The time of exposure must be determined by experiment and will depend on the intensity of the light on the ground glass, the composition of the light before it is filtered, the position of the filter (which should be between the field and substage condensers) and the sensitivity characteristics of the emulsion used. Test exposures should be made with each filter in the following manner: For the red light exposure, the slide is withdrawn and an exposure of one second is given; the slide is inserted part way across the plate, and another exposure of one second is made; the slide is pushed farther in and two seconds' exposure given and again one of four seconds, doubling each time. In this way, a series of strips is formed on the negative, the successive total exposures being: one, two, four, eight, sixteen, thirty-two, etc., seconds. The same procedure is employed with the green filter combination save that here it is advisable to start with fifteen seconds, as the light through these filters is much less intense. These plates, after being developed simultaneously, may be examined and the exposures giving comparable and satisfactory densities selected. If objects of a gray color appear in the section, these should have the same rendering in both negatives, with correct exposures.

*Development.*—Color negatives should be quite soft with delicate tonal gradation. It is best to develop the pairs of plates simultaneously, using a tank. Of all the developing solutions tried, the Eastman borax developer D-76 is by far the most satisfactory, and this procedure is partly based on the use of this solution.

(Eastman D-76 developer<sup>1</sup> is prepared as follows:

Elon .....	4 Gm.
Sodium sulphite.....	200 Gm.
Hydroquinone .....	10 Gm.
Borax .....	4 Gm.
Water .....	2 liters

Dissolve the elon separately in a small volume of water [125 F.]. Then dissolve approximately one fourth of the sulphite separately in hot water [160 F.]. Add the hydroquinone with stirring until completely dissolved. Then add this solution to the elon solution. Now dissolve the remainder of the sulphite in hot water [160 F.]. Add the borax, and when it is dissolved, add the mixture to the aforementioned solution. Dilute to volume with cold water.)

Exposure is adjusted to give satisfactory density in six minutes at 65 F. (18 C.). The development can be done in total darkness by time and temperature alone or, better, the plates may be given a preliminary desensitizing bath of 1:10,000 pinakrytol green for two minutes. The cage containing the plates is immersed in the desensitizing bath in total darkness for the required time, drained, and then placed in the developer. After about two minutes, a rather bright ruby light can be turned on with no risk of fog, although it is best to give the plates no unnecessary exposure to light. The time and temperature method can thus be supplemented by direct visual examination and underexposure or overexposure partially corrected in development. On completion of development, the cage with the plates can be transferred to the rinsing water and thence to the fixing bath, so that they need not be handled individually until they are ready for the final washing.

*Registration Marks.*—After the negatives are dry, registration marks are placed on them so that the lantern plates may be properly placed in position when printing. A lantern slide cover glass or a spoiled lantern slide plate from which

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1. Eastman Professional Films, Rochester, N. Y., Eastman Kodak Company, p. 17.



Fig. 1—Photomicrograph of a myocardial lesion,  $\times 200$ ; hematoxylin and eosin stain. This print is made from the blue-printing negative, exposed by red light. The black area is the shadow cast by the marking device in the plate-holder. The registration marks have been inked over to give them greater prominence.

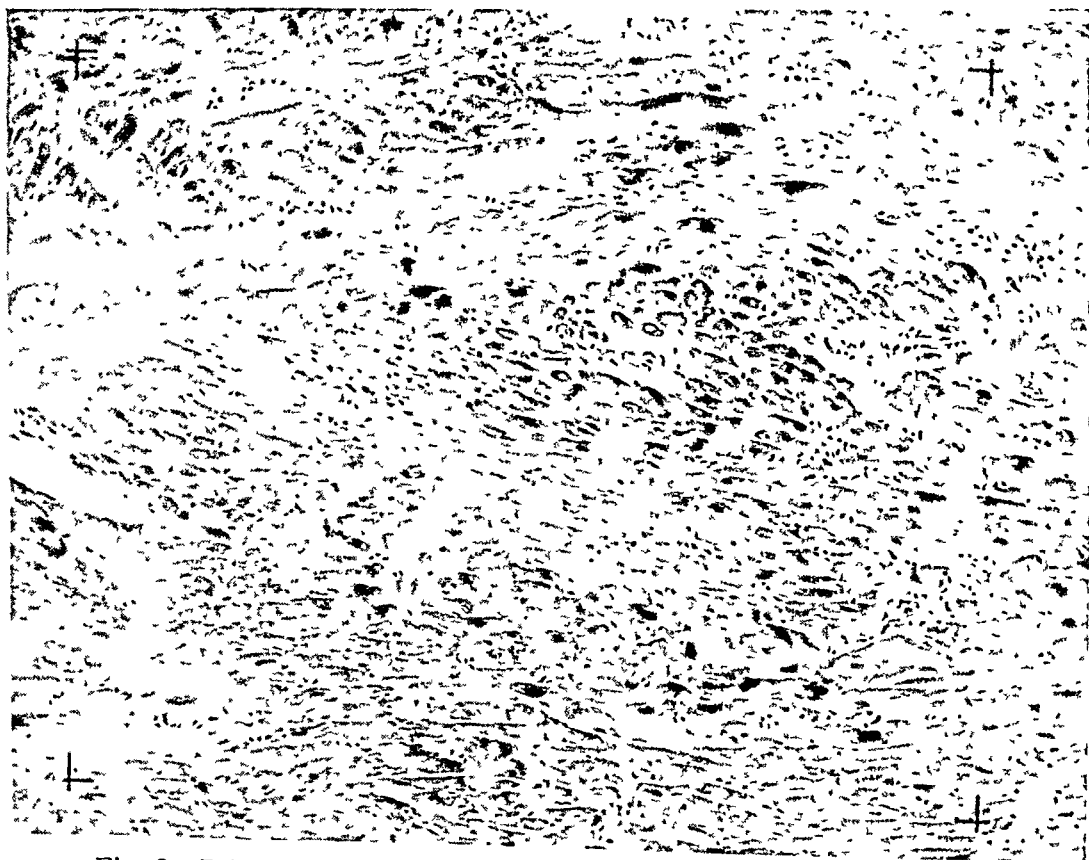


Fig. 2—Print from the red-printing negative of the same area as figure 1. Exposure was made by green light. This negative is also satisfactory for black and white reproductions on paper, or for black and white lantern slides.





the emulsion has been removed is placed over one of the negatives, which is allowed to lie emulsion side up on a glass surface illuminated from below. The lantern plate is adjusted so that the field desired is properly centered, and two points are selected that appear in both negatives and their positions are indicated by tiny dots of ink placed on the registration plate. These points may be nuclei, artefacts or any definite structures that are clearly shown in both negatives.

While the plates are held firmly in position, a sharp scalpel is used to make cuts in the negative emulsion outlining the corners of the registration plate. This is then transferred to the second negative, its position being determined by aligning the ink dots with the previously chosen points in the negative, and similar cuts are made in the emulsion of this negative at the corners of the plate (figs. 1 and 2). In printing, it is necessary only to place the lantern slide plates with their corners coinciding with the intersections of the cuts to obtain close registration, which may be made exact by a very slight shifting of the two positives in mounting.

*Printing.*—Blue Image: The negative made through the red filter is used to make the blue image and should be marked with the word blue, so that confusion will not result in the printing room. The blue image is obtained by first making a *thin* black and white slide in the usual way. By holding the negative up to the ruby light the lantern slide can be accurately placed by means of the registration cuts in the emulsion. The negative and positive plates are put together, emulsion to emulsion, and the exposure made with a 25 watt lamp at a distance of about 10 feet (3 meters). The exposure should be adjusted so that development takes at least two minutes, giving a thin but rather brilliant slide.

After the slide has been fixed and washed, the silver image is toned blue by the iron toning process.

(The iron toning solution T-11<sup>2</sup> is prepared as follows:

Ammonium persulphate.....	0.5 Gm.
Iron and ammonium sulphate (ferric alum).....	1.4 Gm.
Oxalic acid.....	3.1 Gm.
Potassium ferricyanide .....	1.0 Gm.
Ammonium alum.....	5.2 Gm.
Hydrochloric acid 10 per cent.....	1 cc.
Water to make.....	1 liter

Each of the solid chemicals should be dissolved separately in a small quantity of water and the solutions then mixed strictly in the order given, and the whole diluted to the required volume. The bath will be pale yellow and perfectly clear.)

Toning is complete in from five to ten minutes, and there is a moderate intensification; hence, the necessity for having a thin slide with clear whites to start with. After being washed for ten minutes and dried, the blue plate is completed.

Red Image: The negative made through the green filter combination is used to produce the red plate. Here no satisfactory red-toning procedure is available that surpasses the printing on bichromatized gelatin. Ordinary lantern slide plates have a soluble emulsion that is ideal for this method of printing and, in addition, the plates are more uniformly coated than are most of those coated by amateurs. The plates are sensitized by bathing for five minutes in the following solution:

Ammonium bichromate.....	30 Gm.
Strong ammonia.....	5 cc.
Water .....	1 liter

2. Lantern Slides, Rochester, N. Y., Eastman Kodak Company, p. 27.

The temperature of the bath should not exceed 65 F. (18 C.); otherwise the gelatin may develop reticulation. After a quick rinse in distilled water to remove the excess of the sensitizing solution, the plates are dried in the dark in a current of air. When dry, they are sensitive to white light, but may be handled safely in a bright orange light or a subdued artificial light.

With these plates, the printing must be through the back and by the light of the carbon arc. The lantern slide plate is placed with the back in contact with the emulsion of the red-printing negative and its position adjusted by means of the registration marks. Since now the thickness of the plate intervenes between the negative emulsion and the bichromatized gelatin, printing must be done with parallel rays in order that sharp rendition may be obtained. The arc is therefore placed at the focal point of a condensing lens at least as large as the printing frame so that the negative surface makes a right angle with the light rays. The time of exposure will vary from thirty seconds to several minutes, depending on the density of the negative and the intensity of the arc. A fair judgment of the exposure may be obtained by examining the positive plate from the emulsion side. With proper exposure, the image will be fully visible.

Development is accomplished by immersing the exposed plate (emulsion side up) in a tray of warm water at a temperature of about 125 F. (52 C.). With gentle rocking, the unexposed gelatin begins to dissolve in about one minute. Development is continued until the image appears clearly defined and no more gelatin appears to be dissolving. Owing to the silver salt, which here acts as an inert pigment, the image is sharply visualized in all its tonal gradations. If the plate has been underexposed, the high light detail will be lost, while if it has been overexposed, the gelatin will not dissolve sufficiently to make possible a clearing of the high lights. Development will be complete in from five to eight minutes.

Since the silver halide is of no further use, it is removed and the gelatin hardened by immersion in a chrome alum fixing bath. If any particles of sediment form, they may be removed by gently swabbing the plate with a tuft of cotton during washing. After fixation and washing, the plate will appear perfectly clear, and it will be only by using oblique lighting that the image can be perceived. Staining of the image may be done immediately, or the plate may be dried and stained subsequently.

A 1 per cent aqueous solution of eosin is used for staining. If less rapid and somewhat more brilliant staining is desired, the solution may be acidified with acetic acid. In general, moderate overstaining followed by sufficient washing to clear the high lights will be found to give a very satisfactory brilliance. After drying, the plate is ready to be mounted.

Concerning the intensity of staining desired, it is impossible to give explicit directions; only actual experiment will suffice. In the beginning it is well to make the positives in a color intensity series, and by trying various combinations in the lantern the proper balance will be perceived quickly. Subsequent positive pairs are then made in equivalent color intensity.

*Mounting.*—One of the plates is placed emulsion side up on the table and a small drop of hard Canada balsam placed at each of two diagonally opposed corners. A thin, ready-cut mask is placed in position, and two more drops of balsam are placed on the mask at the same corners. The second plate is lowered face down in the proper position for registration. The pack is then lifted and, while held in the fingers, the plates are adjusted until registration is exact. When they are in the correct relationship, the assembly is clamped in a lantern slide-vise, and the edges are bound as usual. Plates bound in this manner will not slip even with rough handling.

## SUMMARY

The foregoing procedure has been developed, not as a unique contribution to the subject of color reproduction, but as a method by which any one with moderate photographic skill can produce excellent lantern slides in color without being forced to purchase expensive specialized materials. Slides prepared in this manner have a high degree of transparency together with precise rendition of fine detail. By relatively slight modifications, the method can be extended to the reproduction of two-color combinations other than red and blue. Finally, only standardized materials, with which the photographer is familiar, are employed.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths.**—William Ophüls, professor of pathology in the school of medicine of Stanford University, San Francisco, has resigned as dean of the school after sixteen years of service.

Julius Klosterman is assistant professor of bacteriology and pathology in New York University.

Howard T. Karsner, professor of pathology in Western Reserve University, has been elected a member for the United States of the executive committee of the International Society for Geographic Pathology.

James H. Peers has been appointed instructor in pathology in the school of medicine of Boston University.

Georg Schmorl, prosector and director of the pathologic institute of the Stadt Krankenhaus Friedrichstadt in Dresden, until April 1, 1932, died on August 14, at the age of 71. While preparing a spinal column he scratched a finger and died of streptococcal septicemia after an illness of eleven days. His favorite subject of study for many years was bone pathology, most recently the vertebrae and intervertebral disks in particular. He had been secretary of the German Pathological Society since 1903.

William B. Brebner, assistant professor of bacteriology, University and Bellevue Hospital Medical College, died on November 9, at 29 years of age, from transverse myelitis, which followed the bite of a monkey.

**New York State Requires Tissue Examinations.**—The state public health council, at a meeting on September 23, added to the sanitary code a new regulation requiring that tissue removed at operation or necropsy for examination must be submitted to an approved laboratory, to the division of laboratories and research in Albany or New York or to the state institute for the study of malignant diseases at Buffalo. The new rule, which applies to all examinations made to aid in the diagnosis, prevention or treatment of disease or to determine the cause of death, takes effect on Jan. 1, 1933.

**Society of American Bacteriologists.**—At the annual meeting in Ann Arbor, Mich., from December 28 to 30, inclusive, the tercentenary of the birth of Anton van Leeuwenhoek will be commemorated by a historical exhibit of Leeuwenhoekiana. Round table discussions will be held on (1) bacterial filtrability, dissociation and life cycles, (2) microbiology of frozen foods and (3) taxonomy.

**Scientific Exhibit, Milwaukee Session of American Medical Association.**—Application blanks are now available for space in the Scientific Exhibit at the Milwaukee Session of the American Medical Association, June 12 to 16, 1933. The final date for filing such applications is Feb. 13, 1933, after which time assignments of space will be made. The Committee on Scientific Exhibit requires that all applicants fill out the regular application form. Persons desiring to receive application blanks should address a request to the Director, Scientific Exhibit, American Medical Association, 535 North Dearborn Street, Chicago.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE RELATION OF HEART WEIGHT TO THE BASAL METABOLISM AS VARIED BY THYROID ADMINISTRATION. F. M. SMITH and E. M. MACKAY, J. Exper. Med. 55:903, 1932.

A linear relationship exists in the albinic rat between the heart weight and the basal metabolic rate when varied by the administration of active thyroid material. It is suggested that this increase in heart weight which follows the increase in metabolism after the administration of thyroid is in the nature of a simple work hypertrophy of the myocardium.

AUTHORS' SUMMARY.

THE ANEMIA OF SCURVY. S. R. METTIER and W. B. CHEW, J. Exper. Med. 55:971, 1932.

Loss in weight, progressive anemia and moderate increase in reticulated red blood cells occurred in seventeen guinea-pigs on a diet deficient in vitamin C. The bone marrow of the guinea-pigs with scurvy showed large numbers of erythro-genic cells, but scant evidence of active maturation to adult erythrocytes. A reticulocyte response was induced in guinea-pigs with scurvy when they were fed orange juice daily. During the reticulocyte response, the bone marrow showed large numbers of mitotic figures and relatively more adult red blood cells than did the bone marrow of guinea-pigs with scurvy that had not been treated with orange juice. It is concluded from this study that the anemia of guinea-pigs with experimentally induced scurvy is largely dependent on a deficiency of vitamin C resulting in retarded maturation of the red blood cells.

AUTHORS' SUMMARY.

BILE PIGMENT AND CHOLESTEROL IN THE GALL BLADDER BILE OF THE DOG. C. RIEGEL, C. G. JOHNSON and I. S. RAVDIN, J. Exper. Med. 56:1, 1932.

The data obtained from the experiments on dogs reported in this paper lead us to conclude that bile pigment is not absorbed from the bile of the gallbladder. The mean loss of pigment is so small when compared with the amount of water lost that it is negligible. Our data on cholesterol do not support the concept that this substance is secreted into the bile of the gallbladder under normal conditions. In the majority of experiments there was a loss of cholesterol. Indeed, we have failed to find any evidence of definite secretion or absorption save in the case of the infected gallbladder. We are led to conclude, as did Rous and McMaster with regard to bile pigment, that normally there is no absorption of cholesterol.

AUTHORS' SUMMARY.

FIXATION BY THE INFLAMMATORY REACTION. V. MENKIN, J. Exper. Med. 56:157, 1932.

The results of this study furnish evidence, in addition to that already provided, that fixation of foreign substances is primarily due to mechanical obstruction by a fibrin network and by thrombosed lymphatics at the site of inflammation. The significance of fixation in relation to immunity and its bearing on some of the other processes involved in the inflammatory reaction are stressed.

AUTHOR'S SUMMARY.

PERMEABILITY OF THE LYMPHATIC CAPILLARY. S. HUDACK and P. D. McMASTER, J. Exper. Med. 56:223 and 239, 1932.

A technic was developed for the demonstration of lymphatic capillaries in the ear of the mouse by means of vital dyes and for tests of their permeability under normal and pathologic conditions. The lymphatics became visible as closed channels from which the dyes escaped secondarily into the tissue. Some of them, cross-connections, with extremely narrow lumens, seemed ordinarily not to be utilized. There was active flow along the lymphatics of the ear of the mouse under ordinary circumstances. The movement of dye was always toward the main collecting system. The valves of the lymphatics as well as the flow of the fluid prevented distal spread. There was, in addition, slow migration, apparently interstitial, but in the same general direction, of dots of color produced by the local injection of dye. The normal permeability of the lymphatics was studied with dyes of graded diffusibility. Their walls proved readily permeable for those highly diffusible pigments that the blood capillaries let through easily, but retained those that the latter retained. Finely particulate matter (india ink, "*Hydrokollag*") they did not let pass. No gradient of permeability was observed to exist along them such as exists along the blood capillaries of certain organs. The observed phenomena of lymphatic permeability, like those of the permeability of the blood capillaries, can be explained on the assumption that the lymphatic wall behaves like a semipermeable membrane.

A standardized solution of a vital dye which escapes with some difficulty from the lymphatics of the ear of the mouse was utilized in tests of the permeability of the lymphatic wall under various conditions. It was found that this permeability is subject to great change. Slight pressure that sufficed to prevent lymph flow from the ear—an organ in which such flow goes on normally—soon resulted in increased permeability of the obstructed lymphatics without any perceptible dilatation of these vessels. Mechanical stimulation—for example, a stroke with a blunt wire or scratching so light as not to break the epidermis—resulted in a practically immediate great increase in lymphatic permeability, which was sharply localized to the region pressed on. This increase in permeability, though so great that even hemoglobin was let pass by the lymphatics, endured but a few hours. Warming the ear to 43 C. or exposure to mild sunlight increased permeability considerably. Slight chemical irritation increased it greatly, though not so much that particulate matter was allowed to pass. The edema developing as a result of lymphatic obstruction or of mechanical, thermal or chemical stimulation was preceded by and associated with a large increase in lymphatic permeability. The facts are discussed in relation to their bearing on the accumulation of fluid within the tissue. It is plain that influences within the realm of the normal suffice to increase lymphatic permeability, and that those which lead to edema cause a very great increase in it. In proportion as this increase occurs, the lymphatics cease to be channels demarcated by a semipermeable membrane. It seems certain that the changes must be in some part responsible for the local accumulation of fluid. There exist possibilities, on the other hand, of a correlation between the functionings of the blood and lymph vessels under certain pathologic conditions, as during the resorption of edema.

AUTHORS' SUMMARIES.

THE INFLUENCE OF AGE ON COMPENSATORY RENAL HYPERTROPHY. E. M. MacKAY, L. L. MacKAY and T. ADDIS, J. Exper. Med. 56:255, 1932.

Compensatory hypertrophy of the kidney in albinic rats becomes less as age advances. There is a rapid decrease at from 5 days to 60 days of age, and then a slow diminution throughout adult life.

AUTHORS' SUMMARY.

THE PRODUCTION OF OSTEOGENIC SARCOMA AND OTHER CHANGES FROM RADIOACTIVE MATERIAL INJECTED INTO RABBITS. F. R. SABIN, C. A. DOAN and C. E. FORKNER, J. Exper. Med. 56:267, 1932.

The observations in this work suggest that, with certain doses of radioactive material, the fundamental damage in the lymphoid tissues is to the stem cell, and

that the damage is to the chromatin of the nucleus of the cell. The erythroid tissues are apparently less susceptible to damage by radioactive material than the lymphoid tissues, but an original anemia of secondary type from peripheral destruction may eventually be changed to one of primary type through decreased maturation of primitive cells in the marrow. The damage of lymph nodes and bone marrow leads to atrophy of these organs. The cells of the liver and thymus suffer nuclear damage of the same general character as is seen in the lymph nodes, and there is atrophy of these organs. The storage of the radioactive material in the bones gave rise to osteogenic sarcomas in two of seven rabbits surviving from eleven to nineteen months. A repetition of the experiment has been undertaken with more intensive studies to test the validity of the findings.

## AUTHORS' SUMMARY.

GEOGRAPHIC PATHOLOGY OF GOITER. C. A. HELLWIG, Surg., Gynec. & Obst. 55:35, 1932.

No modern American treatise on diseases of the thyroid gland refers to the variations in form and function of goiters according to the part of the country in which they occur. From the few facts which are known it would appear that North American goiters do not vary in different regions as widely as do European. The few statistical data available for North America seem to indicate that the goiters observed in this country are rather uniform, but very different from those in regions of high endemicity (Switzerland, Himalayas, Pyrenees). In Kansas and Wisconsin in surgical patients, there are more diffuse than nodular goiters. In North American surgical material, the diffuse and nodular colloid goiters are the most common (from 68 to 79 per cent). Thyrotoxic symptoms, accompanying goiter, are more frequent in this country than in any other so far studied (70 per cent of the surgical cases). For a systematic comparison of the morphology and physiology of goiters in many different areas of North America, the acceptance of a uniform classification appears essential. Aschoff's classification, which, in 1927, at the International Goiter Conference in Bern found the unanimous consent of all leading students of goiter, is recommended.

## AUTHOR'S SUMMARY.

CORTICAL PROLIFERATION IN THE MOUSE SUPRARENAL AFTER PEPTONE. RAYMOND WHITEHEAD, Brit. J. Exper. Path. 13:200, 1932.

Intraperitoneal administration of Witte's peptone in maximal doses to mice was followed by abnormal proliferation in the suprarenal cortex. Mitosis first became appreciable fifty-six hours after dosing, was maximal between seventy-one and eighty-two hours thereafter, and was declining at ninety-seven hours. Mitoses were most numerous in the outer part of the cortex. In its inner part, they were very rare. Dispirems far outnumbered the other phases of mitosis, which in order of decreasing frequency were monospirems, monasters and diasters. Mitosis was as a rule most active in mice showing the least departure from the normal in temperature, general condition and weight. Both morphologic and clinical evidence supports the view that the mitoses seen in the suprarenal cortex after the administration of peptone are probably an indication of active function.

## AUTHOR'S SUMMARY.

EXPERIMENTAL NEPHRITIS PRODUCED BY THE STYRYL QUINOLINE COMPOUND No. 90. H. L. SHEEHAN, J. Path. & Bact. 35:589, 1932.

A styryl quinoline compound, no. 90, produces acute nephritis on injection into rabbits. This is characterized by necrosis of entire first convoluted tubules chiefly in the outer parts of the cortex, followed by regeneration of the tubular epithelium. After about nine days, fibrosis develops around the regenerated tubules, which then gradually atrophy and disappear. The glomeruli remain intact in the resultant scars for about six months and then become fibrosed by a process similar to ischemic atrophy. In those animals that live for over two months after moderately



severe nephritis, the blood urea is permanently slightly raised, but the concentrating power of the kidneys becomes normal. When a harmless dose of no. 90 is given, the dye is taken up directly from the blood stream by the epithelium of the first convoluted tubules and of the broad ascending limbs of Henle. This absorption depends on the physiologic activity of the cells of the tubules and is inhibited by previous damage to them. The manner of excretion suggests that about half of the dye taken up by the cells of the tubules at the beginning is secreted into the lumen of the tubules in the course of the next twenty-four hours; the remainder disappears, possibly as a result of intracellular destruction. When a nephrotoxic dose is given, the process of adsorption and excretion appears to be the same, but the cells are damaged by the large amount of dye which they accumulate in their cytoplasm. The excretion in the urine is decreased in proportion to the extent of the lesions, and there is thus a prolonged retention of this large amount of dye in the cells, which probably continues and completes the initial damage.

AUTHOR'S SUMMARY.

### Pathologic Anatomy

ARTHRITIS DEFORMANS OF THE SACRO-ILIAC JOINTS. F. ZOELLNER, *Virchows Arch. f. path. Anat.* **277**:817, 1930.

Examination of the sacro-iliac joints in sixty cadavers revealed frequently arthritis deformans. Owing to the anatomic peculiarities of this joint, the pathologic picture of the disease is somewhat different from that in other joints. Exulcerations of the cartilage are usually absent. The proliferating vessels of the bone marrow entering the damaged cartilage frequently cause occlusion of the joint space. This is followed later on by cartilaginous adhesion and finally by true ankylosis.

W. SAPHIR.

THE HISTOLOGY OF ACUTE RHEUMATIC FEVER. F. KLINGE, *Virchows Arch. f. path. Anat.* **279**:1 and 16, 1930.

In a previous communication, Klinge described, as the earliest tissue reaction in acute rheumatic infection, fibrinoid degeneration of connective tissue, with edematous dissociation of connective tissue fibrillae and death of connective tissue cells. This process occurs in connective tissue throughout the body and has been noted by him in the tonsils, myocardium, pericardium, endocardium, blood vessels, muscles, tendons, joints and hepatic and splenic capsules. The reaction is a focal one. In two consecutive papers, he now describes the intermediate or subacute stage of the reaction and the terminal stage. The primary, degenerative stage of tissue reaction is followed by proliferation of connective tissue, with the formation of the characteristic rheumatic granulomatous nodule. This, too, is a temporary stage in the rheumatic tissue reaction. In time the cells of the nodule degenerate and disintegrate, the replacing connective tissue differentiates, and the nodule becomes transformed into a scar, which in rare instances may undergo calcification or ossification. Klinge attempts to differentiate between clinical and anatomic recidives in rheumatic infection. By clinical recidive he understands an exact repetition of the three stages in the pathologic process after an interval of freedom from the disease. By anatomic recidive he understands the development of more recent reactions in the scars of older lesions or the presence of all three stages of the pathologic process in the same case.

W. SAPHIR.

ORIGIN OF THE MIGRATORY CELLS OF ACUTE INFLAMMATION. F. FREUND, *Virchows Arch. f. path. Anat.* **279**:30, 1930.

In the acute traumatic inflammation of the tongue of the living frog, in the experiment devised by Cohnheim, the presence of many large wandering cells is noted. These, according to Freund's observations, do not come from the blood but are derived from the connective tissue.

W. SAPHIR.

FACTORS INFLUENCING LEUKOCYTIC EMIGRATION IN ACUTE INFLAMMATION. M. NORDMANN and A. REUTLER, *Virchows Arch. f. path. Anat.* **279:45**, 1930.

The observations reported were made on the mesentery of the living rabbit infected with colon bacilli. The parietal or marginal localization of the leukocytes within the vessels, which is a prerequisite for leukocytic emigration, is dependent on retardation of the blood flow and decreased excitability of the local vasoconstrictors. Leukocytic emigration can be suppressed by preventing retardation of the blood flow by means of drugs that cause local vasodilatation, such as cocaine or atropine, or by causing a general hypertension through elimination of the mechanism regulating blood pressure.

W. SAPHIR.

DIFFUSE HEMANGIOMATOSIS OF THE SPLEEN. A. A. WASSILJEFF and A. W. PROTASSEWITSCH, *Virchows Arch. f. path. Anat.* **279:79**, 1930.

Cavernous hemangioma of the spleen may occur as a single cavernoma, as multiple cavernomas and as diffuse hemangiomatosis. The last named is the rarest, only a few examples being recorded in the literature. The authors' example occurred in a man, aged 32, who had an enlarged spleen and no significant laboratory findings. Splenectomy was done, and the patient left the hospital in good condition in three weeks. The large, congested spleen revealed a few pea-sized cavities. Microscopically, the spleen was transformed into hemangiomatous cavities of variable size. They contained a colloid-like material, little blood and deposited iron and calcium salts. The spaces were surrounded by and separated from each other by thick walls of connective tissue. The authors believe that the colloid contents of the spaces were derived from the contained blood.

W. SAPHIR.

VASCULAR GRANULOMAS OF THE PLEURA SIMULATING ECCHYMOSES. G. KOHLSCHUETTER, *Virchows Arch. f. path. Anat.* **279:85**, 1930.

Subpleural ecchymoses of larger or smaller size are frequently seen when death is due to passive congestion, suffocation or acute sepsis. Such ecchymoses may be confused with lesions of similar gross appearance, but of entirely different histologic structure. The lesions that Kohlschuetter describes occur as bluish or black, slightly elevated, circular or elliptic spots of variable size. They may be few in number and then are usually located near the interlobar fissure, or they may be numerous and scattered over the entire visceral pleura. They are composed of granulation tissue containing numerous capillary blood vessels and mononuclear lymphoid cells, the latter often aggregated about the vessels. Blood pigment or other evidence of previous hemorrhage has never been seen by Kohlschuetter. He believes that the lesions arise as the result of minute ruptures of the pleura due to spells of coughing caused by chronic bronchitis. Prolapse of pulmonary tissue through the minute ruptures occurs, and this is followed by reparative inflammatory reactions, which lead to the formation of the lesions described. Because of their similarity to subpleural ecchymoses, they may have medicolegal importance.

W. SAPHIR.

EXPERIMENTAL REGENERATION IN TISSUE CULTURE. A. FISCHER, *Virchows Arch. f. path. Anat.* **279:91**, 1930.

Regeneration in tissue culture bears a close resemblance to that of healing wounds. Fischer made wounds in tissue cultures by excising a central area or a sector from the periphery. Regeneration of tissue immediately set in at the margin of the wound. The speed of the regenerative process was independent of the nutritive conditions of the culture, but was proportional to the age of the culture. The number of mitoses at the margin of the wound was identical with that at the growing periphery of the culture. The speed of the regenerative growth was

increased as compared with that of the control culture. This was probably due to substances that developed after the wound had been induced, and that were capable of promoting growth. This explanation is supported by the fact that tissue cultures subjected to repeated wounding grew at a markedly greater rate than the control cultures.

W. SAPHIR.

INTESTINAL LYMPHOGRANULOMATOSIS. W. NOWICKI, *Virchows Arch. f. path. Anat.* **279**:146, 1930.

Nowicki gives a thorough review of the literature of primary isolated lymphogranulomatosis and presents a case of his own. A man, aged 36, died with symptoms of intestinal obstruction. Necropsy showed stenosis of the ileum, due to an infiltrative thickening of the wall that narrowed the lumen. The tissue had the histologic characteristics of cellular lymphogranulomatous tissue. No other evidences of the disease elsewhere could be detected.

W. SAPHIR.

HISTOLOGY OF FOLLICULAR KERATOSIS (DARIER'S DISEASE). H. HAMLI, *Virchows Arch. f. path. Anat.* **279**:237, 1930.

This study of the chronic dermatosis described by Darier and named by him "dyscratose folliculaire" is based on material removed for biopsy. The characteristic alteration is hyperplasia of the basal cell layer of the epidermis, with lateral and downward ingrowth into the cutis. Concentric masses of the proliferated epithelium may become isolated in the cutis, forming the "corps ronds" of Darier, termed perloid bodies by Hamli. The clinical manifestations are due to extension in various directions and to regressive and regenerative, healing processes. In agreement with Andry, Hamli prefers the descriptive designation "pseudofollicular" to "follicular," since in all the lesions examined he has never seen primary involvement of the follicles.

W. SAPHIR.

ACANTHOSIS NIGRICANS WITH INVOLVEMENT OF THE ESOPHAGUS. H. TESSERAUX, *Virchows Arch. f. path. Anat.* **279**:244, 1930.

In a woman with the clinical diagnosis of carcinoma of the stomach, acanthosis nigricans of the skin developed three months before death. At necropsy there was found a carcinoma of the stomach, with metastasis to the regional lymph nodes. A circumscribed papillary lesion of the mucosa of the esophagus revealed, histologically, papillary hypertrophy of the submucosa, with round cell infiltration and marked thickening of the epithelial layer. The histologic picture was similar to that of the acanthotic lesions of the skin.

W. SAPHIR.

OBLITERATION OF THE THORACIC DUCT. A. FEHR, *Virchows Arch. f. path. Anat.* **279**:265, 1930.

In a woman, aged 50, with chylous ascites and chylous hydrothorax, necropsy revealed carcinoma of the stomach. The lymph vessels of the region were obstructed by carcinomatous thrombi. The thoracic duct was obliterated in the greater portion of its course. The obliteration was due to a condition termed by Fehr endolymphangitis productiva, which he believes was secondary to the carcinomatous obstruction of the radicles of the duct. Obstruction here was followed by proliferation of the intima of the main duct and by thrombosis within the narrowed lumen.

W. SAPHIR.

MUCOUS CYSTS OF THE KNEE JOINT. P. ZAECH-CHRISTEN, *Virchows Arch. f. path. Anat.* **279**:273, 1930.

Mucous cysts of the knee joint are rare. From a review of the literature and a study of four cases of his own, Zaech-Christen concludes that these cysts

arise from the outer border of the semilunar cartilage. Histologically, they are identical with the ordinary ganglion. The cysts are filled with a mucous fluid and are lined by a layer of flat cells of endothelial character. The cysts probably arise from the para-articular mesenchymal tissue, which proliferates as the result of irritation or stimulation. The proliferated tissue undergoes mucoid transformation, and in time a cavity is formed. Such cysts are not tumors, but are inflammatory malformations.

W. SAPHIR.

### Microbiology and Parasitology

DERIVATION OF STAPHYLOCOCCUS ALBUS, CITREUS AND ROSEUS FROM STAPHYLOCOCCUS AUREUS. M. PINNER and M. VOLDRICH, J. Infect. Dis. **50**:185, 1932.

Apathogenic *S. albus*, *S. citreus* and *S. roseus* are split off spontaneously from pure line strains of pathogenic *S. aureus*. The splitting-off of *S. albus* can be much enhanced by growing *S. aureus* in broth containing *S. aureus* agglutinins and by animal passage. The three nonidentical strains derived from *S. aureus* appear to be stable under ordinary conditions. They differ from the mother strains in regard to pigment production, growth intensity, individual morphology, staining reactions, virulence, fermentative and proteolytic action, hemolysin production, alkali production, agglutinability, immunizing properties and susceptibility to bacteriophage. From *albus* strains *aureus* strains can be recovered by culturing the *albus* strains for prolonged periods in mediums containing high concentrations of anti-*albus* serum. The relation between *aureus* and *albus* strains is essentially similar to that between S and R strains of other micro-organisms.

AUTHORS' SUMMARY.

THE BACTERIOLOGY OF BRONCHIECTASIS. P. H. GREY, J. Infect. Dis. **50**:203, 1932.

A uniform bacterial flora was not found in nine cases of bronchiectasis. Bacteria of various types were obtained in each of the cases studied. The absence of spirochetes in five of these cases suggests that their presence in the other four was a chance invasion. This also holds true for the other micro-organisms isolated. Pure cultures of a single type of streptococcus have been obtained from the bronchi following lobectomy on three occasions, which indicates that a mixed infection is not necessarily present. The variety of micro-organisms recovered suggests that no specific type plays an etiologic rôle, but that many bacteria are capable of producing bronchial damage sufficient to favor dilatation.

AUTHOR'S SUMMARY.

THE VIRUS OF HERPES. M. HOLDEN, J. Infect. Dis. **50**:218, 1932.

The enhanced resistance of desiccated herpes virus to deterioration, heat and the action of certain chemicals is striking when compared with the resistance of moist herpes virus to the same factors. A comparison of the resistance of pneumococci, both moist and dried, to chemical agents and heat, with the resistance of herpes virus under similar conditions, furnishes results that are not inconsistent with the assumption of the living nature of the virus.

AUTHOR'S SUMMARY.

THE EFFECT OF URINE ON HEMOLYTIC STREPTOCOCCI. G. H. ROBINSON and F. A. TAYLOR, J. Infect. Dis. **50**:249, 1932.

Dissociation of hemolytic streptococci by exposure of the organisms to urine is doubtful, or at least of rare occurrence, and cannot be controlled. Hemolytic streptococci grow poorly in urine, while indifferent streptococci grow abundantly. Hemolytic streptococci live longer in a medium composed of the inorganic salts of urine alone than in one in which the normal amount of urea is added to the

saline constituents. The nondialyzable constituents of urine are not suitable for the support of growth of the hemolytic variety, but suffice for the growth of the indifferent organisms for a considerable time.

AUTHORS' SUMMARY.

EFFECT OF IODINE ON THE TUBERCLE BACILLUS AND EXPERIMENTAL TUBERCULOSIS. G. KNAYSI, *J. Infect. Dis.* **50**:255 and 261, 1932.

The results of the present experiments show that iodine is toxic to the tubercle bacillus cells, for these cells were unable to grow on slants after thirty minutes' contact with as high a dilution of iodine as 1:10, or to infect guinea-pigs after an exposure of fifteen minutes to iodine in the dilution 1:5  $\times$  10. My results are contrary to the report of DeWitt and Sherman that compound solution of iodine is not able consistently to kill the tubercle bacillus cells even after an exposure of twenty-four hours. The results obtained by these investigators become understandable if one remembers that they used cell clumps in their test tube experiments and dried cells in their experiments on animals. When a clump of cells is placed in an iodine solution, the iodine reacts with the lipoids of the cells on the outside, and this slows down its diffusion to the interior of the clump. On the other hand, it is a fact that the cells become less permeable on drying. For instance, they stain less readily, and it becomes difficult to demonstrate satisfactorily in dried cells intracellular substances such as fat and glycogen without a relatively long application of the reagent. The effects of clumping and drying may be put in evidence by studying the diffusion velocity of iodine in strips of filter paper, wet and dry, and with and without previous imbibition of an emulsion of lecithin. It will be found that iodine diffuses most slowly in a strip of filter paper that has been immersed in an emulsion of lecithin and allowed to dry, less slowly if the lecithin-treated paper has not been dried, faster in a dry, untreated strip, and fastest in a strip wet with distilled water.

Iodine may be injected intravenously into the body of the rabbit over relatively long periods of time without apparent harm to the health of the animal or injury to the thyroid gland. The dose used in most of these experiments was 2 mg. per kilogram of body weight, administered twice a week over a period of about two months, from an aqueous solution containing three times as much of potassium iodide. The results show that such a small dose is not effective in the treatment of tuberculosis, probably because it is quickly neutralized by the alkali reserve of the blood. A dose of 30 mg. of iodine per kilogram of body weight does not kill a normal animal, but one of 20 mg. per kilogram is fatal to a rabbit with severe miliary tuberculosis. This subject deserves further consideration under more appropriate conditions.

AUTHOR'S SUMMARIES.

THE INFLUENCE OF BLOOD AND OF EXUDATE ON THE COLON BACTERIOPHAGE. M. APPLEBAUM and W. J. MACNEAL, *J. Infect. Dis.* **50**:269, 1932.

Purulent exudate, even when diluted, exerts an interfering influence on the lytic action of bacteriophage against the colon bacillus, provided that the colon bacillus is capable of active growth in the medium employed. Human blood serum and rabbit blood serum exert a similar but less powerful interfering influence, which becomes evident when pathogenic strains of the colon bacillus recently obtained from human infections are employed. Whole blood produces an effect similar to that of serum. Suspensions of erythrocytes in an indifferent medium seem to exert little, if any, influence on the bacteriophage phenomenon in relation to the colon bacillus. These observations have a bearing on the use of bacteriophage in the treatment of infectious disease.

AUTHORS' SUMMARY.

PURIFICATION OF THE VIRUS OF VACCINIA. C. A. BEHRENS and L. B. MORGAN, *J. Infect. Dis.* **50**:277, 1932.

Two methods, (a) the iso-electric and (b) the aluminum gel, have been developed for the purification of the virus of vaccinia. The iso-electric and the

aluminum gel methods are both satisfactory for neurovirus emulsions, but only the aluminum gel method is applicable to the dermavirus emulsion.

AUTHORS' SUMMARY.

BLOOD CULTURE IN RHEUMATIC FEVER. L. E. COOLEY, J. Infect. Dis. 50:330, 1932.

Blood cultures from twenty-five children with rheumatic fever remained sterile for one month, except for an occasional contaminant. The positive results obtained by others were not confirmed.

AUTHOR'S SUMMARY.

RAT-BITE FEVER ASSOCIATED WITH SPOROTHRIX. N. P. ANDERSON and B. K. SPECTOR, J. Infect. Dis. 50: 344, 1932.

A typical case of rat-bite fever is reported, the evidence indicating that a sporothrix-like organism played a part in the attack. As far as we know, this is the first time that such an organism has been isolated from a patient suffering from rat-bite fever.

AUTHORS' SUMMARY.

DISSOCIATION IN STREPTOCOCCUS SCARLATINAE. E. DELVES, J. Infect. Dis. 50: 350, 1932.

The streptococcus of scarlet fever was dissociated into three main colonial types, S, SR and R. The S colonies were small, smooth and conical, grew diffusely in broth and were opsonically specific. The SR colonies were granular on the surface, with an even but irregular outline, and grew in broth with some sedimentation, although the supernatant medium was slightly cloudy. The R colonies were rough-surfaced, with fimbriated margins, and gave a clumpy growth in broth, the supernatant medium remaining clear. Neither the SR nor the R colonies were opsonically specific, but reverted strains regained this specificity. The S culture when stained was composed of short chains of small cocci. The stained SR chains were of medium length and composed of large cocci, while the R cultures showed long chains of large cocci with flattened opposing surfaces. The Gram reaction became more variable as the dissociation proceeded from S to SR to R. The virulence tests, while the results were not clearcut, owing to the fact that streptococci lose virulence on an artificial medium, would indicate that S was more virulent than R. Reversion in the animal body to forms that were able to cause abscesses, as well as reversion by serial transplants in an artificial medium, was observed. Dissociation of S to SR to R was also noted in infusion immune serum broth of  $pH$  7.8, while neutral broth was not as effective.

AUTHOR'S SUMMARY.

DISSOCIATION OF STREPTOCOCCI. L. E. COOLEY, J. Infect. Dis. 50:358, 1932.

Six different strains of streptococci associated with rheumatic fever and arthritis were grown on chocolate agar for several months. Five of the strains showed definite evidences of spontaneous dissociation, two strains dissociating to the R form, while three remained as the SR form. In young colonies of one of the R forms, definite bacilli were seen. These bacilli later divided to form the adult irregular coccoid forms. The other R form showed no bacillary types during its growth. Coccobacilli and short, thick bacilli were seen in two of the SR forms during their period of growth. One of the R forms was more virulent for mice than the S form of the same strain. There was no difference in virulence for mice between the S, SR or R forms of the other strains. All of the dissociated strains were repeatedly brought back to the original S form by being grown in dextrose broth medium.

AUTHOR'S SUMMARY.

EXPERIMENTS ON A FILTRABLE PHASE IN THE LIFE HISTORY OF THE TUBERCLE BACILLUS. R. R. MELLON, *Tubercle* **13**:10, 1931.

Employing filtrates of tuberculous material from sixteen different sources, Mellon reports that in two of thirty-seven animals classic tuberculosis resulted, and that the typical Koch bacillus was recovered in pure culture. These positive results were obtained only when the original material was richly seeded with the granular form of the tubercle bacillus. Histologic evidence of tubercles in guinea-pigs is not of itself sufficient proof of filtrability, since such lesions can be caused by gram-negative paratyphoid-like micro-organisms. The relation of this group of micro-organisms to lesions indistinguishable from the necrobiotic type of tubercles in guinea-pigs must always be kept in mind.

H. J. CORPER.

THE ETIOLOGY AND PATHOGENESIS OF LYMPHOGRANULOMATOSIS. TITU VASILIU, *Ann. d'anat. path.* **8**:815, 1931.

From a review of the literature and from personal studies, Vasiliu concludes that tuberculosis is often mixed with lymphogranulomatosis, at times conspicuously, but at other times so intimately that one can hardly detect its presence on microscopic examination. Animals susceptible to tuberculosis become tuberculous in a high number of instances when inoculated with lymphogranulomatous material whether an infection with acid-fast bacilli was or was not found in the latter.

On the other hand, in a number of instances, lymphogranulomatosis associated with tuberculosis produces no lesions (in the rabbit and guinea-pig) of the customary tuberculous type. Nevertheless, tissues from animals thus infected yield cultures of Koch's bacilli on inoculation in artificial mediums. Again, cases were reported in which tissues showing no acid-fast micro-organisms produced tuberculosis in animals on reinoculation.

Lesions closely resembling those of lymphogranulomatosis were produced with lymphogranulomatous material, particularly with that associated with tuberculosis.

Filtrates obtained from tubercle bacilli have sometimes caused lesions resembling very much those of lymphogranulomatosis. Tubercle bacilli have very rarely caused such lesions.

Although Vasiliu is of the opinion that the etiology of lymphogranulomatosis is not established, he does consider that the relationship between the tubercle bacillus and lymphogranulomatosis is not entirely eliminated.

B. M. FRIED.

SECONDARY BRONCHOGENIC ECHINOCOCCIC CYSTS OF THE LUNG. F. DÉVÉ, *Ann. d'anat. path.* **9**:77, 1931.

Most uncomplicated hydatid cysts of the lung are fertile when they rupture, and in addition to the daughter cysts, innumerable scolices are dispersed throughout the bronchial tree. Contrary to the opinion of certain authors, these scolices are capable of producing secondary cysts as Dévé demonstrated experimentally in 1904. The experiments have been repeated, and a series of photomicrographs shows the various steps in the dissemination, encystment and growth of the scolices. In the literature, five cases of multiple echinococcic cysts are described, which can be explained as due to secondary implants following rupture of a primary cyst into a bronchus.

ALBERT F. DE GROAT.

RABIES. P. REMLINGER and J. BAILLY, *Ann. Inst. Pasteur* **47**:608, 1931.

The article, apparently the first of a series, presents various phases of the activity of the virus. The virus was often demonstrated in the spleens of various infected animals, most frequently in those of cats (seven of fifteen animals). Liver and kidney also yielded the virus. Certain areas in the nervous system were occasionally encountered in which there was no virus. A study of five fixed viruses confirmed the idea that these vary in virulence and in symptoms produced. Frogs and toads were found to be completely refractory. "The passage of fixed virus

into the central nervous system of vaccinated animals is no more a question of strain than of the method of treatment or of the animal used." Dilution was found not to attenuate the virus. A review of four cases in which repeated vaccination was practiced (in one, four times in four years) indicated that no untoward reactions should be expected.

M. S. MARSHALL.

SUBACUTE INGUINAL LYMPHOGRANULOMATOSIS. C. LEVADITI, P. RAVAUT, P. LÉPINE and R. SCHOEN, *Ann. Inst. Pasteur* 48:27, 1932.

The disease appears to be due to a specific virus, "the lymphogranulomatous virus," present in the inguinal glands during this disease, which, on intracerebral injection into monkeys induces typical inflammatory changes in the meninges, paralysis and, on some occasions, death. The virus was not found other than in cases of this disease. The most virulent of strains isolated was, after twelve passages through monkeys, still virulent for man (used in a case of dementia paralytica). The virus is filtrable, loses its pathogenicity after heating at 60 C. for thirty minutes, is stable in glycerin, does not resist drying, resists antiseptics well, except formaldehyde 1:1,000, and carries a negative charge. Monkeys and mice were successfully inoculated; rabbits, rats and cats were not susceptible. Silent infections and other variations in virulence were noted. The histologic picture was characterized by infiltration of the meninges, extensive perivascular lymphoma, morphologic integrity of nerve cells and necrotic areas in human ganglions. Photographs and colored plates of these changes are given. Inoculation in the inguinal nodes induced bilateral inguinal adenitis, from which the virus was recovered ten weeks after inoculation. Inoculation of the prepuce was also followed by adenitis, a tendency to ulceration followed by spontaneous resorption, inguinal and axillary polyadenitis and tumefaction. The affinity for the nervous system is less marked than that of some other viruses. The infection is generalized, since liver, kidney, spleen and bone marrow yielded the virus, although blood inoculations failed. Monkeys surviving intraganglionic inoculation are refractory to intracerebral inoculation, and serum from patients with the disease neutralized the virus in vitro, although vaccination proved difficult.

M. S. MARSHALL.

TUBERCULOUS ULTRAVIRUS. G. SANARELLI and A. ALESSANDRINI, *Ann. Inst. Pasteur* 48:144, 1932.

The ultravirus in collodion sacs introduced into the peritoneal cavity of the guinea-pig induced death due to "tuberculosis of the Calmette-Valtis type"—adenitis, slight enlargement of the spleen, acid-fast elements, but no tubercles. Two or three serial transfers through guinea-pigs resulted in typical experimental tuberculosis, with tubercles and acid-fast rods. Tubercle bacilli in collodion sacs implanted in the animal induced only the picture of an infection with the ultravirus. Prolonged exposure, or infection, still gave the same anatomic changes, without tubercles. The acid-fast elements derived from infected guinea-pigs showed attenuated pathogenicity. The ultravirus exhibited lymphotropic properties—an affinity for lymph nodes and serous cavities—with proliferative inflammatory processes, hyperplastic or caseogenous, and giant cells, but no tubercles. On the second animal transfer, the spleen usually showed a few nodules; subsequent transfers resulted in increasing dissemination. Cultivation of tubercle bacilli from the infected animal is difficult, even in early animal passage in which bacillary forms appear. Colonies appear only after prolonged incubation, in keeping with the conception of poor vital energy on the part of ultravirus granules.

FROM THE AUTHORS' CONCLUSIONS.

EXPERIMENTAL TRACHOMA. UGO LUMBROSO, *Arch. Inst. Pasteur de Tunis* 20:253, 1931.

After reviewing the conflicting reports with regard to the rôle of *Bacterium granulosis* in trachoma, Lombroso proceeds to discuss further experimental work



in which he used his types A, B and C, bacteriologically described in a previous article in the same periodical. Granulation was produced in one of four species of monkeys tested with A, indicating a refractory state on the part of the common *Macacus cynomolgus* and other species. Apparent infection with type B was indicated in several species of monkeys; a failure was attributed to the strain of culture used. When *M. cynomolgus* only was used, natural granulation of the conjunctiva was noted in animals inoculated with true type C, but the strains were considered avirulent. Representative type strains were injected into two series of human subjects (seven in all), with results that indicated, as in the experiments on animals, that type A is rarely pathogenic, that type B is particularly virulent, and that type C never induces infection.

M. S. MARSHALL.

SILENT INFECTION OF MAN BY THE VIRUS OF DISTEMPER. CHARLES NICOLLE, Arch. Inst. Pasteur de Tunis **20**:321, 1931.

The question why there are a number of infections transmitted from animal to man, but none from man to animal, is raised. The virus of distemper was injected subcutaneously into a man and into a monkey. Although of proved virulence, it caused no symptoms, but the blood of the human subject reinjected into rigidly quarantined dogs induced the infection. Nicolle reasons that man is susceptible to infection with the virus in the *forme inapparente*; that man may thus act as a reservoir for some diseases of animals, although the fact is not recognized, and that when two species are susceptible to a virus, one typically and the other without apparent symptoms, the disease is older in the second species.

M. S. MARSHALL.

MEXICAN TYPHUS. CHARLES NICOLLE, Arch. Inst. Pasteur de Tunis **20**:324, 1931.

Nicolle is unequivocal in stating that Mexican typhus and the European disease are the same. Clinical differences are secondary: more extensive eruption, less significant cerebral and cardiac symptoms, less severe disease even in immigrants, with more rapid convalescence, and a much greater tendency to develop hemorrhages, especially epistaxis. The viruses of Europe and Mexico are identical on cross-protection tests. In the guinea-pig, the Mexican virus induces an earlier fever of shorter duration and orchitis, which is not produced by the Tunisian virus. The latter shows a cerebral localization. The white rat and gray rat have a silent infection with the Tunisian virus; in Mexico the black rat was found to act as a reservoir of the infectious agent. The systematic relationship between Mexican typhus, the typhus of valleys of the Andes and European typhus is briefly considered.

M. S. MARSHALL.

BACILLUS PROTEUS AND TYPHUS. F. LECHUITON, Arch. Inst. Pasteur de Tunis **20**:444, 1932.

Bacteriophage-lysed filtrates of *B. proteus* X 19 failed to induce typhus in guinea-pigs, nor did they produce immunity to typhus. Similar results obtained with formaldehydized filtrates and also with strains placed in contact with the brain tissue of animals with typhus under a variety of conditions. Strains that had lost the property of agglutinability did not recover it when in contact with the brain tissue of infected guinea-pigs or with the blood of infected monkeys.

FROM AUTHOR'S CONCLUSIONS.

GLANDERS IN A WOMAN. W. A. AFANASSJEW, Centralbl. f. allg. Path. u. path. Anat. **53**:148, 1931.

Glanders, apparently starting in the nose, in a 50 year old peasant woman resulted in the production of pea-sized nodules in the skin of the nose and face,

right lung and spleen. The nodules were exudative rather than neoplastic. In the sublobular veins of the liver were emboli of liver cells.

GEORGE RUKSTINAT.

ACUTE SEPTIC AVIAN TUBERCULOSIS IN A MAN. H. NASSE, *Centralbl. f. allg. Path. u. path. Anat.* **53**:209, 1931.

The body of a 53 year old metal-worker was entirely covered with a finely speckled, red-brown exanthem; each lung had about fifty pinhead-sized yellow nodules just beneath the pleura; nodules varying in size from that of a millet seed to that of a hazelnut were found in the liver, spleen, epiglottis, bowel and kidney, and hazelnut-sized caseous masses were found in the tracheobronchial lymph glands. The nodules contained clumps of tubercle bacilli, nuclear débris at their centers and many monocytes. Lymphocytes and polymorphonuclear leukocytes predominated; epithelioid cells were scarce and giant cells entirely absent. Coupled with the histologic picture in these nodules, which was typical of avian tubercles, was a history that the deceased person had eaten many raw eggs. Nasse cites the startling estimate that 11 per cent of chickens in Germany are tuberculous, and that from 3 to 7 per cent of eggs on the market contain tubercle bacilli.

GEORGE RUKSTINAT.

TUBERCLE BACILLEMIA IN TUBERCULOSIS OF THE SKIN. F. KOCH, *Deutsche med. Wchnschr.* **58**:90, 1932.

With Löwenstein's culture method for detecting tubercle bacilli in the blood, Koch failed to obtain any positive results in twelve cases of lupus vulgaris, two of annular granuloma, two of indurated erythema, four of lupus erythematosus, four of verrucous tuberculosis of the skin and one of tuberculous epididymitis. He cannot offer any explanation why his results should differ so widely from those obtained by Löwenstein.

ENTEROCOCCUS ENDOCARDITIS. GERHARD ROSENBERG, *Klin. Wchnschr.* **11**:359, 1932.

The clinical progress and the results of a postmortem examination in a case of aortic and mitral endocarditis in which the infecting organism was an enterococcus of type B are reported. The duration of the infection was ten weeks. There were embolic mycotic abscesses and perivascular scars of the myocardium, and infarcts of the spleen. The abscesses differ from the lesions of endocarditis lenta (Schottmüller), caused by *Streptococcus viridans*. EDWIN F. HIRSCH.

SEPSIS DUE TO BACILLUS INFLUENZAE. F. E. KOCH, *München. med. Wchnschr.* **79**:706, 1932.

In a girl, aged 3½ years, with sepsis caused by *B. influenzae* and a diphtheroid infection of the throat, there were found after death bilateral confluent bronchopneumonia, pleuritis, fatty changes of the heart and liver, and marked edema of the brain. The blood gave a pure growth of *B. influenzae*.

EDWIN F. HIRSCH.

TAPEWORM OF THE HUMAN GALLBLADDER. E. OFFER, *Virchows Arch. f. path. Anat.* **279**:193, 1930.

Offer says that his is the third recorded case of tapeworm infestation of the human gallbladder. The patient was a woman, aged 52, who died rather suddenly with symptoms of acute gallbladder disease. Necropsy revealed acute generalized purulent peritonitis, which was secondary to ulcerative and phlegmonous cholecystitis with partial necrosis of the wall. The gallbladder was filled with purulent bile, which contained a tapeworm (*Taenia saginata*) 25 cm. long. There were no calculi.

W. SAPHIR.

FILTRATES OF CULTURES OF STAPHYLOCOCCI. HANS GROSS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:14, 1931.

The hemolyzing, necrotizing, leukocidal and toxic properties of filtrates of certain cultures of staphylococci are manifestations of the same antigenic substance. They are all present in potent filtrates, show identical thermoresistance and are all absorbed by antisera. The substance promoting clotting which is found in some filtrates differs qualitatively from those previously mentioned.

I. DAVIDSOHN.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE. S. HELLERSTRÖM and E. WASSÉN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:110 and 114, 1931.

Intraperitoneal injection of this virus produced peritonitis with a characteristic cellular exudate. The latter contained the virus, which was also demonstrated in the blood and in the spleen. The virus passed through the scarified skin of monkeys and infected them without producing a local reaction. The virus passed Berkefeld and Chamberland filters, was quickly destroyed by glycerin ( $p_H$  5) and resisted freezing for at least twenty-two days.

I. DAVIDSOHN.

A NEW STRAIN OF CHICKEN LEUKOSIS. J. ENGELBRETH-HOLM, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:126, 1931.

Leukosis found in a Plymouth Rock chicken was transmitted for ten generations. Blood and suspensions of organs were used successfully for intravenous, intramuscular and subcutaneous injections. Attempts at alimentary transmission did not succeed. The period of incubation was shortened and the frequency of "takes" increased with repeated passages. The opinion of Furth is confirmed that lymphatic leukosis in chickens is a nontransmissible disease *sui generis*, occurring spontaneously and not related to myeloid and erythroleukotic forms.

I. DAVIDSOHN,

THE INFECTIOUSNESS OF PERSPIRATION IN SYPHILIS. IMMO KÖHNE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:279, 1932.

Rabbits that received injections of the perspiration of syphilitic patients did not become infected. No infection resulted when the testicles and popliteal lymph nodes of these rabbits were injected into a second group of rabbits. No spirochetes were detected in the perspiration in the dark field. When spirochetes were added to the perspiration, the mixture produced an infection in the rabbit, although the motility of the spirochetes was quickly inhibited after the addition of the perspiration.

I. DAVIDSOHN.

THE NATURE OF THE BACTERIOPHAGE. Z. W. JERMOLJEW, I. S. BUJANOWSKAJA and W. A. SEVERIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:360, 1932.

Chemical analysis of filtered bacteriophage revealed that it is not a protein or a nucleoprotein, and that it consists of particles smaller than those of albumin. The nonprotein nature was confirmed by means of cataphoresis. It could be produced from dead bacterial bodies. It is concluded that the nature of bacteriophage is not that of a living substance.

I. DAVIDSOHN.

## Immunology

ANTIGENIC RELATIONSHIP OF SHIGELLA DISPAR. H. WELCH and F. L. MICKLE, J. Infect. Dis. **50**:524, 1932.

A Flexner diagnostic serum contains an agglutinin for the dispar type, the titer of which is at least 50 per cent of the titer of the major agglutinins. It is

demonstrated by agglutination and agglutinin absorption that the organisms responsible for dispar agglutinins in the Flexner diagnostic serum are the Strong 2 (Army Medical Museum) and Flexner 6 (Army Medical Museum) strains. Eight of the fifty-three strains studied were capable of absorbing dispar agglutinins from Flexner diagnostic serum. Emphasis is placed on the diagnostic value of recognizing the dispar antigenic component of the Flexner 6, the Strong 2 and particularly the V strain used as stock Flexner in European laboratories. It is suggested, in spite of the discrepancies in fermentative reactions, that neither the dispar nor the Sonne types should be excluded from the Flexner series until further work is brought forward to substantiate the value of such a procedure.

## AUTHORS' SUMMARY.

AGGLUTININ-ABSORPTION STUDIES ON BRUCELLA. W. N. PLASTRIDGE and J. G. McALPINE, *J. Infect. Dis.* **50**:555, 1932.

Agglutinin-absorption data obtained on 142 strains of *Brucella* of human, bovine, porcine, caprine and equine origin are presented. A comparison of the identifications of these strains by their ability to utilize dextrose and their behavior on Huddleson's dye plates with their serologic identifications shows that the agglutinin-absorption test failed to differentiate between *Br. abortus* and *Br. melitensis* so far as twenty-three, or 15 per cent, of the strains studied were concerned. In general, the results obtained confirm the opinion expressed by Burnet, namely, that while it is possible to classify some strains of *Br. melitensis* as such by means of the agglutinin-absorption test, it is impossible to identify strains of *Br. abortus* by means of this test with any degree of certainty. Evidence of variation in the serologic properties of the members of the genus *Brucella*, especially *Br. melitensis*, under ordinary laboratory conditions has been noted and discussed from the standpoint of explaining the lack of agreement in the results reported by various investigators.

## AUTHORS' SUMMARY.

SKIN REACTIONS TO *NECATOR AMERICANUS* IN PERSONS INFECTED WITH THE COMMON INTESTINAL PARASITES. G. W. BACHMAN and R. RODRIGUEZ-MOLINA, Porto Rico *J. Pub. Health & Trop. Med.* **7**:287, 1932.

For practical application of the intracutaneous test in detecting the presence of *Necator americanus* in persons infected with two or more intestinal nematodes, the measurable characteristics are too variable, and the reactions are not significantly specific.

## FROM AUTHORS' SUMMARY.

CROSS-IMMUNITY BETWEEN SOUTH AFRICAN TYPHUS AND TICK-BITE FEVER. ADRIANUS PIJPER and HELEN DAU, *Brit. J. Exper. Path.* **13**:33, 1932.

The virus of South African typhus confers immunity in guinea-pigs against the virus of tick-bite fever. The virus of tick-bite fever, however, does not confer immunity in guinea-pigs against South African typhus.

## AUTHORS' SUMMARY.

THE SEROLOGICAL DIAGNOSIS OF TYPHOID AND PARATYPHOID FEVERS. J. SMITH, *J. Hyg.* **32**:143, 1932.

An analysis of the results of serologic study of blood specimens submitted during the past two years for the Widal test has been made. The serums from persons with typhoid and paratyphoid fever were examined in particular for their content of agglutinin for the H and O antigenic constituents of *Bacillus typhosus* and *Bacillus paratyphosus* B. The results indicate that, from the point of view of early diagnosis it is essential to use the O-antigen of *B. typhosus*, but that no particular advantage is derived from the use of the O-antigen of *B. paratyphosus*. A comparison of the value of suitable living and killed antigens has been made. The two types of antigen appear to be equally effective, but for convenience and

stability the suspensions issued by the Standards Laboratory, Oxford, have proved entirely satisfactory. Cases showing the serologic difficulties that arise when a mixed specific and nonspecific form of the H-antigen of *B. paratyphosus* B is used are described.

AUTHOR'S SUMMARY.

ANTICOMPLEMENTARY SERA. R. D. MACKENZIE and R. S. MARSHALL, *J. Path. & Bact.* **35**:175, 1932.

An anticomplementary action has been noted in the serums of natives of Northern Nigeria. This action is present in the serums of almost all the persons with sleeping sickness whom we have examined, and to a lesser degree in the serums of those suffering from other conditions. It is thermostable and cannot be removed by filtration. It has no relation to the amount of natural complement present in the serum. Its significance is not understood. It is strong enough to interfere with the carrying out of complement-fixation tests by the usual methods.

AUTHORS' SUMMARY.

### Tumors

GANGLIONEUROMA. J. A. BIGLER and A. HOYNE, *Am. J. Dis. Child.* **43**:1552, 1932.

Ganglioneuromas are rarely reported in children. The diagnosis is difficult, as the symptoms are usually due to pressure on adjacent tissues or organs. Two cases are reported: In one, a mediastinal ganglioma caused compression and displacement of the trachea, with laryngeal or tracheal obstruction. In the other, a ganglioma at the upper pole of the right kidney was discovered at necropsy. Microscopically, these tumors were true benign ganglioneuromas. Following a review of the literature a classification of the reported cases is outlined on the basis of the schematic diagram of the development of the sympathetic nervous system by Landau and modified by Schultz. Three main types of tumors develop from the formative cells of the sympathetic nervous system: neuroblastomas from neuroblasts, chromaffinomas or paraganglionomas from the pheochromoblasts and ganglioneuromas from the adult ganglion cells. Intermediate or mixed types may occur. The neuroblastoma, which is the most common type in children, arises frequently in the suprarenals, resembles a small cell sarcoma, and metastasizes early to the lymph nodes, the bones and the liver. The rare chromaffinoma is small and seems to be benign. The ganglioneuroma is considered ripe and benign when early formative cells are not present. The ripe ganglioneuroma is rare in children; it grossly resembles a fibromyoma but microscopically possesses a characteristic appearance. Ganglioneuromas which contain early formative cells may ripen and become benign or may metastasize.

RALPH FULLER.

ALEUKEMIC, MYELOGENOUS CHLOROMA. LEONARD A. SWANSON, *Am. J. Dis. Child.* **44**:140, 1932.

A case of chloroma occurring in a girl, 2 years of age, and terminating in death within sixty-eight days after the onset of symptoms has been described. Profound anemia was present when the patient was first examined, twenty-three days after the onset of symptoms, and progressed in severity to the time of death. Roentgenograms of the long bones taken a month after the onset of the disease did not indicate a generalized involvement of the bone marrow. Repeated examination of the blood failed to disclose any leukemic changes. The tumor was primary in the bones of the skull, with the early production of exophthalmos, and metastases were present in the lymph nodes, ovaries and suprarenals. Both the primary and the secondary tumors gave a strongly positive reaction to oxydase. The characteristic green color was obscured in the primary tumors by necrosis and hemorrhage, but was typical in the metastases. Roentgen therapy was instituted within a month after the onset of symptoms, with no beneficial effects.

AUTHOR'S SUMMARY.

MULTIPLE EPITHELIOMAS ORIGINATING FROM CONGENITAL PIGMENTED NEVI.  
R. NOMLAND, Arch. Dermat. & Syph. **25**:1002, 1932.

A case, apparently unique in the literature, is described in which epitheliomas arose in adult life from pigmented basal cell nevi present since birth. The patient was a woman 38 years old.

HISTOLOGIC CLASSIFICATION OF TUMORS OF THE CENTRAL NERVOUS SYSTEM.  
G. ROUSSY and C. OBERLING, Arch. Neurol. & Psychiat. **27**:1281, 1932.

On the basis of studies of 251 cases, Roussy and Oberling offer a simplified classification of cerebral tumors into five main types: glioma (arising from neuroglia), ependymochoroid neoplasms (arising from the ependymal lining including that of the choroid plexus), ganglioneuroma (arising from the ganglion cells), neurospongioma (originating from neuroblasts and spongioblasts) and neuro-epithelioma. Each group is subdivided; for instance, glioma is subdivided into astrocytoma, oligodendrocytoma and other types of tumor. Of the 251 tumors studied, 178 were gliomas, 26 ependymomas and the rest neurospongiomas, ganglioneuromas and unclassified tumors.

GEORGE B. HASSIN.

HISTOLOGIC DIAGNOSIS OF TUMORS OF THE BRAIN. PERCIVAL BAILEY, Arch. Neurol. & Psychiat. **27**:1290, 1932.

Bailey recognizes three great families of primary tumors of the brain: medulloblastoma, multiform glioblastoma and astrocytoma. They differ in age of onset, site of origin and biologic behavior. Medulloblastoma, for instance, is almost exclusively a cerebellar tumor in children. Glioblastoma, also described as multiform spongioblastoma, occurs almost exclusively in adults, in the cerebral hemispheres, while astrocytoma is the classic glioma, a slowly growing tumor. Groups less frequently observed are comprised of oligodendroglioma (relatively benign), unipolar and bipolar spongioblastoma, especially the type that grows in the vicinity of the optic chiasm, astroblastoma, ependymoma and pinealoma, ganglioneuroma and neuro-epithelioma. Bailey states that perhaps 12 or 15 per cent of the gliomas cannot be affiliated with any of the foregoing groups, and that the classification is not only of academic, but also of practical importance.

GEORGE B. HASSIN.

TUMORS OF THE SHEATHS OF THE NERVOUS SYSTEM. WILDER PENFIELD, Arch. Neurol. & Psychiat. **27**:1298, 1932.

The primary tumors of the meninges are classified by Penfield as meningeal fibroblastoma, otherwise known as dural endothelioma, meningioma and psammoma; meningeal sarcoma, melanoblastoma, lipoma and other types. To tumors of the nerve sheaths the author refers perineurial fibroblastoma, otherwise known as neurinoma, and peripheral glioma which the author proposes to call schwannoma, for, he thinks, they originate from the Schwann cells or their precursors.

GEORGE B. HASSIN.

INTRACRANIAL CHORDOMA. HERMAN SELINSKY, Arch. Neurol. & Psychiat. **28**:413, 1932.

Chordoma in the region of the pituitary body produces the usual signs and symptoms of increased intracranial pressure, but no manifestations of dispituitarism. This feature was noted in Selinsky's patient, who complained of progressively failing vision and various ocular disturbances (hemianopsia and palsies of various optic muscles). Necropsy revealed a chordoma, the size of a plum, over the sella, which was destroyed.

GEORGE B. HASSIN.

AN OSTEOLASTIC OSTEOID TISSUE-FORMING TUMOR OF A METACARPAL BONE.  
HENRY L. JAFFE and LEO MAYER, Arch. Surg. 24:550, 1932.

A tumor of the fourth metacarpal bone was described. It began when the patient, a girl, was 12 years of age. After growing slowly for almost three years, it began to grow rapidly. The tumor mass was removed; it measured 10 by 6.5 by 6 cm. Only the articular head of the metacarpal bone remained. The tumor was of such consistency that it could be cut with a knife, but it was granular and firm. Histologically, it showed a marked production of osteoid tissue and also the formation of trabeculae of normal, newly formed bone. The osteoid tissue arose from cells that had many of the features of osteoblasts. The tumor has not recurred two years after operation, although on casual examination the histologic appearance suggests the diagnosis of osteogenic sarcoma. It is believed that this tumor is an osteoblastic, osteoid-forming tumor of slow growth, and that the prognosis is favorable. Early in the course of the growth a biopsy was made. The histologic pictures of the biopsy specimen and the surgically removed specimen are practically identical.

The only other report in which tumors of similar nature have been described is that of Bergstrand under the title of "Ueber eine eigenartige, wahrscheinlich bisher nicht beschriebene osteoblastische Krankheit in den langen Knochen der Hand und des Fusses" (*Acta radiol.* 11:597, 1930).

H. L. JAFFE.

SOME FEATURES OF GLIOBLASTOMA MULTIFORME. E. M. DEERY, Bull. Neurol. Inst., New York 2:157, 1932.

The overgrowth of vascular elements found in glioblastoma multiforme has been studied at necropsy in a series of ten cases. Some vessels showed an overgrowth strictly limited to the endothelial cells. Other vessels presented an unaltered intima, but the adventitial elements had undergone hyperplasia. Less frequently, a combination of both endothelial and adventitial overgrowth was found to occur in the same blood vessel. These vascular reactions are not strictly specific, as they may be found in a lesser degree in other conditions, including other types of tumor. The distribution, type and degree of hyperplasia of the vascular elements vary greatly, and large areas in every tumor may contain only normal vessels. In the endothelial form of overgrowth the endothelial cells themselves appear to elaborate the heavy reticulin-collagen framework which is characteristic of this type of reaction. Necrosis, so common in glioblastoma multiforme, is probably caused as much by focal toxic factors as it is by the decrease in blood supply. Large numbers of phagocytic cells are produced by the adventitial tissues of the vessels bordering an area of necrosis. The majority of these phagocytes eventually become indistinguishable from the gitter cells of microglia.

AUTHOR'S SUMMARY.

THE BLOOD SUPPLY OF THE GLIOMAS. C. A. ELSBERG and C. C. HARE, Bull. Neurol. Inst., New York 2:210, 1932.

In the gliomas of the brain there is a close connection between the distribution of the blood vessels and the manner in which they infiltrate the brain tissue. On gross and microscopic postmortem examination, the differences between the number of blood vessels in the central and the peripheral parts of the tumors and the number of those in the adjacent brain tissue are striking. In the astrocytomas and the medulloblastomas, the largest number of vessels occur in the central part of the growths, and there is no increase in the number of arteries in the adjacent white matter. In the glioblastoma multiforme, the peripheral areas of the neoplasm contain the largest number of arteries, and the vessels in the adjacent tissue of the brain are more numerous than in normal white matter or in the part of the brain which is adjacent to the margins of the astrocytomas and the medulloblastomas. As there is a close connection between blood supply and active growth, it has been possible to gain some insight into the manner in which the astrocytomas,

medulloblastomas and glioblastomas increase in size and infiltrate the brain which surrounds them. The glioblastoma multiforme is a rapidly growing and malignant tumor, and in the final analysis, the "active" infiltration of the brain and the peripheral type of growth are due to its malignancy, which is the major factor. The differences in blood supply and in the manner in which the brain is invaded in the astrocytoma and the medulloblastoma, on the one hand, and in the glioblastoma multiforme, on the other, are expressions of the varying degrees of malignancy. The difference in the situation of the blood vessels between the astrocytoma and medulloblastoma and the glioblastoma multiforme can be recognized at operation, and this has led us to modify and to alter to some extent the technic used for the removal of such growths. In order to gain an approximate idea of the vascularity of different parts of the growth and of its size, the suggestion is made that at operation the tumor should be bisected before an attempt to remove it is made. This procedure will permit the surgeon to orient himself better regarding the size and removability of the growth. If he is dealing with an astrocytoma or a medulloblastoma, the central, most actively growing part should be removed first. The excision of the peripheral parts may be simplified by this technic. In the glioblastoma multiforme, on the other hand, the periphery of the tumor should be removed with especial care.

AUTHORS' SUMMARY.

VENOUS AND ARTERIOVENOUS ANGIOMAS OF THE BRAIN. S. BROCK and C. G. DYKE, *Bull. Neurol. Inst., New York* 2:247, 1932.

Eight instances of intracranial angioma are described. In four, extracranial vascular lesions coexisted. An unusual instance of venous angioma of the retina, chiasm, midbrain and cerebellum is described. The important optic signs were homonymous hemianopia and unilateral exophthalmos. In the arteriovenous variety the cardiovascular phenomena were of diagnostic significance. They included enlargement of the intracranial, cranial, extracranial and carotid arteries and of the heart; a systolic mitral murmur and a mild degree of tachycardia. A low systolic pressure with a much reduced diastolic blood pressure and a Corrigan pulse were sometimes found. The resemblance of the cardiovascular disturbances to those seen in aortic regurgitation is commented on. An arterial bruit of extracranial or of intracranial origin is an important sign, too often overlooked. The significant roentgenographic observations consisted of intracerebral calcifications, and dilatation and tortuosity of the vascular grooves in the bones of the skull. The peculiar character of the calcification found in the venous angiomas was pathognomonic.

AUTHORS' SUMMARY.

PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR. J. B. MURPHY and E. STURM, *J. Exper. Med.* 56:483, 1932.

Water extracts of desiccates of certain relatively slow-growing strains of chicken tumors I and X, or the exudates from such tumors, definitely inhibited the growth of a mouse sarcoma and were without effect on a mouse carcinoma or mouse tumor S/37, a rapidly growing sarcoma derived from the stroma of a carcinoma. Extensive control tests with extracts from rapidly growing chicken tumors, and from tissues of normal and immune chickens showed no inhibiting action. There was no demonstrable action on the mouse tumors of serums from immunized rabbits, which neutralize the chicken tumor agent, or of the serums from chickens highly immune to the chicken tumors.

AUTHORS' SUMMARY.

MEDULLO-EPITHELIOMAS OF THE BRAIN AND RETINA. T. B. DAVIE, *J. Path. & Bact.* 35:359, 1932.

A medullo-epithelioma of the brain and two of the retina are described as examples of a subdivision of glioma and as examples of undifferentiated growth rather than of dedifferentiation.



THE BIOLOGY OF NEOPLASTIC CELLS. ALBERT FISCHER, Ann. d'anat. path. 8:665, 1931.

This article by the leading continental student of tissue cultures is a condensed, scholarly résumé of the progress of oncology as a result of the study of tissues *in vitro*. His statements can be summarized as follows:

The classification of isolated cells in histologic sections is of no importance, because the topography of the cellular elements, not their form, is the essential in their identification. On the contrary, the culturing of tissue has taught the investigator to discriminate isolated cells according to their morphology. The method of tissue culture should not be regarded as a particular department, but merely as another method acquired by experimental medicine. Until recent days the study of cancer was in a state similar to that of the study of infectious diseases before the bacterial era. The technic of measuring the respiratory metabolism of neoplastic tissue and the culturing of tissues has enabled one to investigate the physiology of the cancerous cells.

The malignancy of neoplastic tissue has its basis in a specific cancerous cell. One is not yet in a position to differentiate qualitatively between cancerous and normal cells. A method of culturing cells in conditions closely resembling those of the living organism has yielded precious data concerning the processes of regeneration and differentiation of normal cells.

The mechanism of illimited growth of neoplastic cells has become elucidated. The knowledge is based on measuring of the rapidity of growth and on the experiments performed by culturing together normal and neoplastic cells. The mutual influence between these two tissues could be observed with remarkable results.

It is of interest that colonies of cells the growth of which was very slow begin rapidly to proliferate as a result of insults caused to them by minute scratches (*petites blessures*).

Neoplastic cells possess a low resistance, and their life is short. There occurs a continuous destruction of neoplastic cells in tissue cultures.

In conclusion: the limitless proliferation of neoplastic cells in the organism is the physiologic result of the death of the malignant cells just as physiologic regeneration occurs as a result of damage to normal cells. B. M. FRIED.

CARCINOIDS OF THE SMALL INTESTINE. G. N. MARANGOS, Beitr. z. path. Anat. u. z. allg. Path. 86:48, 1931.

In two of four cases of carcinoid of the intestine, there were metastases. All had a desmoplastic tendency, which contributed to the intestinal thickening and angulation. In all cases anaplasia was absent. Serial sections revealed the plexiform structure of carcinoids, and in all nodules above 2.5 mm. invasion of the walls of blood vessels with sheetlike spread beneath the endothelium was found.

W. S. BOIKAN.

ENDEMIC HEMANGIOMATOSIS OF CHICKENS. J. SAL, Virchows Arch. f. path. Anat. 279:62, 1930.

Many of the chickens of a chicken farm near Budapest suddenly became ill. Most of the fowls affected were from 6 months to 1 year old. The disease, which was fatal, was characterized by the development of soft, papillary, easily bleeding tumors at different parts of the skin. Necropsy revealed similar tumors in the liver, heart and lungs. Histologically, the tumor varied from cavernous hemangioma to hemangiosarcoma composed of cellular young tissue between the blood spaces. The sarcomatous tissue infiltrated and invaded the surrounding tissues.

W. SAPHIR.

SYNOVIAL SARCOMA OF THE KNEE JOINT. P. PRYM, *Virchows Arch. f. path. Anat.* **279**:71, 1930.

Prym presents a detailed clinical and pathologic study of the case and reviews three others recorded in the literature. The synovial membrane was transformed into cellular tumor tissue with the morphology of spindle cell sarcoma. On the surface of the membrane were numerous papillary outgrowths covered by a single layer of high cylindric cells.

W. SAPHIR.

CLINICAL AND ROENTGENOLOGIC OBSERVATIONS OF THE JOACHIMSTHAL CANCER OF THE LUNG. A. BEUTEL and A. WOLDRICH, *Ztschr. f. Krebsforsch.* **34**:109, 1931.

In this report on the clinical and roentgen findings in the occupational pulmonary cancers of the Joachimsthal miners, there is included a series of roentgenograms of successive stages of the disease. Beutel and Woldrich conclude that there are two possibilities in the way of origin—either in a bronchial stem of the first order, with a form so flat as not to appear in the x-ray photograph, and with local consolidation representing a nonspecific pneumonia or spread of the cancer; or in a peripheral bronchial stem, with growth centrally and secondary involvement of the larger bronchi. They believe the latter to be the more probable mechanism.

H. E. EGGERS.

IS CHIMNEY-SWEEP'S CANCER REALLY EXTINCT? DUETSCHKE, *Ztschr. f. Krebsforsch.* **34**:159, 1931.

Although chimney-sweep's cancer is becoming so rare in Germany as to be virtually extinct, a recent case of this occupational cancer is reported.

H. E. EGGERS.

### Medicolegal Pathology

INTERNAL CEREBRAL HEMORRHAGE DUE TO ACCIDENT. S. SHELDON, *Brit. M. J.* **1**:332, 1932.

A boy, aged 17, was knocked down by an automobile while he was crossing a road. He was picked up immediately and rushed to a nearby hospital while unconscious, where he died three hours later. There were abrasions on the backs of both hands and the fronts of the knees, a simple fracture of the middle third of the right tibia with much bruising, no scalp wounds or fracture of the skull, a little blood-stained fluid in the subdural space at the base of the brain, flattened convolutions, and an absence of injury or tearing of the outer surface of the brain. On sectioning the brain, a typical laceration with hemorrhage was found on the right side between the caudate nucleus and the optic thalamus, with free blood in both lateral ventricles. The ruptured vessels was on the right side. The remaining vessels were normal and free from atheroma.

WILLIAM FREEMAN.

SEVEN CASES OF GENERALIZED ARGYRIA. GERNEZ, HOUCKE and CUVELIER, *Ann. de méd. lég.* **4**:251, 1932.

A veritable epidemic of argyria was observed by the authors in patients suffering from pulmonary tuberculosis treated with collargol. The usual symptoms of nausea, vomiting, anorexia, abdominal pain and diarrhea accompanied the intoxication. These symptoms continued for several months after discontinuance of the injection of collargol. Silver was not found in the urine or in the blood, but it was present in the hair and in the nails of one patient. Chemical analysis of 10 cc. of bile did not reveal silver, but spectroscopic examination of this material

yielded positive results. The coloration developed after a latent period of about one year, during which time there were no other symptoms except digestive disturbances and progressive asthenia.

H. S. THATCHER.

PECULIARITIES OF MARKS OF CLOTHING ON LEAD BULLETS. H. PIÉDELIEVRE, P. ÉTIENNE-MARTIN and G. PEIFFER, *Ann. de méd. lég.* **7**:477, 1932.

Bullets were placed on cloth held in front of a hard body and struck with a light blow from a hammer. Perfect imprints of the cloth were obtained, photographed and enlarged. These experiments were the controls. Bullets were then shot into a target of certain tissues in front of a block of paraffin. From these experiments the authors conclude that in principle a lead bullet takes the imprint of the first material which it traverses. It is able in certain cases to take the imprint of the second material, which is superimposed. The imprint of the first material happens to be modified also by the mixture with the others. Errors of identification are made, if one ignores the possibility of such a phenomenon.

H. S. THATCHER.

ANATOMIC INFERIORITY OF THE HUMAN ORGANS. W. A. NADESHDIN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:426, 1932.

A study was made of the specific gravity of various organs with a benzene-chloroform mixture, in order to determine the differences between normal, pathologic and senile tissues. Acute poisonings, which selectively affect the blood vessels (cyanide, carbon monoxide, etc.), lower, for instance, the specific gravity of the aortic wall; sclerotic processes increase it. In poisonings, in general, the specific gravity of the parenchymatous organs usually appears lowered. The rigor mortis does not change the specific gravity of the muscle tissues. Since one finds in the kidneys 12 per cent blood, in the spleen 12.5 per cent and in the liver 29 per cent, these organs exhibit an increase in specific gravity in instances of death due to fatal hemorrhage. Tissues that have been preserved in a 10 per cent solution of formaldehyde do not materially differ from those just removed from the dead body.

E. L. MILOSLAVICH.

DETERMINATION OF BLOOD GROUPS IN CADAVERS. V. M. PALMIERI, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:446, 1932.

Group-specific properties of human blood do not disappear immediately after death, but persist for a certain period of time. The intensity of the cadaveric changes and the degree of temperature are important factors. The most satisfactory results are obtained up to the fourth day after death, provided the temperature at which the body is kept does not exceed 22 C. After the fifth day, the possibility of obtaining reliable results decreases gradually, especially if the temperature is high. In one instance, in which the body was kept for twenty days at a temperature of from 8 to 15 C., the blood group was satisfactorily determined. The average longest interval of time at higher degrees of temperature is, in general, not more than eight or nine days. Urinalyses for the group-specific properties constantly showed a negative result. In the pericardial fluid the agglutinins are detectable for a longer period of time, but they disappear faster than those in the serum. A few days after death, the red blood corpuscles lose their form and structure, and the receptors gradually vanish within from ten to fifteen days. The iso-agglutinins of the serum, however, persist at the same temperature for a longer time and are not demonstrable after from the fifteenth to the twentieth day. In old and disintegrated serum, new unspecific agglutinins, so-called panagglutinins, are formed, as the result of bacterial action, and may lead to false interpretations. Should the erythrocytes be nonusable, an examination of the serum should be attempted, employing test erythrocytes A, B and O in lecithin suspensions.

E. L. MILOSLAVICH.

ACUTE POISONING WITH ZINC CHLORIDE. G. PFEIFFER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:1**, 1932.

Up to the present, sixty-two instances are recorded in the literature, with a mortality of 60 per cent, death occurring on the day of poisoning in 40 per cent of the cases. The eschars exhibit a white-gray color, and the mucous membranes show a leather-like, shrunken appearance. The gross changes are somewhat similar to those observed in silver and mercury poisonings. The eschars may remain attached for a long period of time, and the sloughing process is not accompanied by a suppurative demarcation as in alkali poisonings. Later on, the local corrosive action of zinc chloride causes a very pronounced shrinkage of the affected structures, since this poison has the property of deeply and rapidly penetrating the tissues. An extensive shrinkage of the stomach often follows in nonfatal cases, and pyloric stenosis and anacidity are common complications. In contrast to other corrosive poisons, zinc chloride rarely gives rise to perforation of the stomach. If the poison was ingested, signs of corrosion in the mouth, pharynx and esophagus are frequently absent. The longer the victim survives, the more marked are the inflammatory changes in the kidneys. The clinical picture does not contain any symptoms characteristic of zinc chloride poisoning. Violent vomiting and hypersecretion are caused by severe acute gastritis.

E. L. MILOSLAVICH.

CHANGES IN THE PAPILLARY MUSCLES OF THE HEART IN ILLUMINATING GAS POISONING. W. RADTKE, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:26**, 1932.

In poisonings by illuminating gas, characteristic changes are found in the papillary muscles of the left ventricle of the heart, consisting of irregularly distributed areas of hemorrhages, inflammatory reactions and necrosis. These lesions are chiefly met with in the apical portion of the muscles, but may also involve their basal segment. The papillary muscles of the tricuspid are not affected. These findings are observed in cases of a protracted course of carbon monoxide poisoning, being most pronounced if death followed twenty-four hours, or longer, after the exposure. From a medicolegal point of view, these changes in the papillary muscles are of great practical significance, since in a case of death from an obscure cause their detection at autopsy will direct attention to carbon monoxide exposure, as illustrated by two very interesting observations.

E. L. MILOSLAVICH.

TEARING OF THE INTIMA OF THE COMMON CAROTID CAUSED BY STAB WOUND OF THE NECK. J. FAZEKAS, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:54**, 1932.

At autopsy in the case of a 52-year-old man who committed suicide, three stab wounds of the neck were found. The instrument used was a blunt pocket knife. The left common carotid showed, 4 cm. below its bifurcation, a transverse tear in its intimal surface, 6 mm. in size, while the artery otherwise appeared not perforated. The instrument had caught in the corner of the carotid bifurcation and had distended the vessel upward and backward, causing the intima to tear transversely below the actual point of acting force. A transverse intimal tear, as in death by hanging, is the result of a force that elongates or longitudinally distends the blood vessel, while compression alone causes axial or longitudinal laceration of the intima.

E. L. MILOSLAVICH.

SIGNIFICANCE OF FOREIGN MATERIAL IN THE INTESTINES OF THE NEW-BORN INFANT IN PROVING INFANTICIDE. K. BEOETHY, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:58**, 1932.

If a dead body is submerged, water or other fluid mediums may arrive in the stomach, but will not pass the pyloric barrier, except in instances of abnormally

high pressure. Presence of water or other fluid with foreign material suspended in it, below the pyloric ring, is a positive indication that the person was submerged while still alive. Experiments performed on twelve dead bodies of newborn infants proved this important fact.

E. L. MILOSLAVICH.

SWALLOWING OF FOREIGN BODIES BY IMPRISONED CRIMINALS. R. GOEDEL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:63**, 1932.

Nineteen cases are described and psychologically analyzed, disclosing persons of hysterical and psychopathic character, only one fourth of them expressing suicidal intent. In 50 per cent of the cases, repeated ingestion of foreign bodies was observed. Evasion of disciplinary punishment or dodging of assigned prison work was the common driving motive.

E. L. MILOSLAVICH.

ATTEMPTED SUBSTITUTION OF ANOTHER PERSON IN PATERNITY BLOOD TEST.

A. LAUER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:79**, 1932.

The father of the child tried to deceive the expert by sending to the laboratory in his stead a friend for the blood testing ordered by the court. Since in the laboratory every person to be examined is photographed for the purpose of record, a deception was readily prevented. It is suggested that, as a routine, fingerprints also be taken of all persons subjected to this examination of the blood in order to eliminate any possible error or imposition.

E. L. MILOSLAVICH.

DISSIMULATION OF A SEVERE GUNSHOT WOUND OF THE HEAD. W. LESCHMAN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:82**, 1932.

With suicidal intent, an innkeeper shot himself in the right temporal region, at first with no ill effects. He kept the incident secret and continued his regular work for two days and then called a doctor because of grip, coughing and headache. The doctor incidentally discovered the wound, which was concealed under a small piece of adhesive tape. On account of increasing intracranial pressure, trephining was done on the third day, and crushed brain tissue and blood clots exuded. Stereoscopic pictures disclosed the bullet on the opposite side within the skull. Recovery followed.

E. L. MILOSLAVICH.

SEXUAL PERVERSIONS LEADING TO FATAL ACCIDENTS. F. SCHWARZ, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **19:85**, 1932.

Accidental death may unexpectedly occur by interference of unforeseen or unintentional incidents during acts committed because of a perverted sexual instinct. Often the findings at the scene of the accident are baffling and may then be easily misinterpreted and a murder assumed. Certain cases of death by hanging are not suicides but are accidental deaths, as the victim tried, by the act of hanging, to stimulate and increase his sexual orgasm. A young man while sitting in a bathtub at night was looking at some obscene pictures and got hold of a defective electric lamp placed on a chair nearby. Electrocutation caused death. The lamp was found submerged in the bathtub, and the man's lungs showed some aspiration of water. A second case involved a young person with transvestitistic perversion, who was found dead in his bedroom dressed only with ladies' stockings and a garter supporter showing dried sperm spots. Various articles of women's underwear were neatly stored in his dresser. Death occurred from carbon monoxide poisoning due to an open jet of a gas stove, the flame of which had been put out by the stormy wind blowing through an adjoining chimney flue.

E. L. MILOSLAVICH.

# Society Transactions

## BUFFALO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Nov. 4, 1932*

KORNEL TERPLAN, *President, in the Chair*

### STUDIES OF PLASMA PROTEINS. J. LOESCH.

The relationship between changes in the plasma proteins and damage to the kidneys produced in dogs in two different ways was studied. Ischemia of the kidneys produced by clamping the renal pedicle was followed by a rise in the plasma fibrinogen. This was noted when both kidneys were treated in a normal animal or when one kidney was manipulated six months after a unilateral nephrectomy. This effect was not obtained during the terminal condition, when a marked retention of nitrogenous products had taken place.

I believe that the increase in fibrinogen was due to stimulation of the liver, and that the decrease in the terminal condition was a result of impaired function of the liver brought about by the retained waste material.

If the renal pedicle was not manipulated, animals from which as much as three quarters had been removed showed no change in plasma fibrinogen after the immediate effects of operation had passed. The only change noted in this group of dogs was an increase in the plasma albumin, which was not great in any instance, but was regularly observed when diets high in protein were fed. As this increase paralleled slight variation in the cells it seemed probable that it was caused by decreased hydration of the blood.

### RECURRING JAUNDICE WITH REPORT OF BIOPSY. S. SANES.

Several days following a gastro-intestinal upset consisting of nausea and emesis, abdominal cramps and diarrhea, deep jaundice developed rather acutely in a white woman, aged 38. Six months previously, the patient had received an intravenous injection of arsphenamine (0.2 Gm.) at three weekly intervals; no untoward symptoms were experienced. Physical examination revealed, in addition to jaundice, only a palpable liver. No evidences of syphilis were demonstrated. The temperature ranged from 99 to 99.5 F. Laboratory studies made at various times during the patient's illness yielded information as follows: icteric index, from 100 to 75; van den Bergh test, immediate reaction positive, from 62 to 6 units; bromsulphalein test, 100 per cent retention after thirty minutes; urobilin in urine, from 2 plus to 0; Wassermann reaction, negative; duodenal drainage, bile-positive; leukocyte count, not significant; culture, no pathogenic bacteria. In the fifth week of her illness, the patient was seized with a severe pain in the upper part of the abdomen accompanied by emesis. Laparotomy disclosed the liver and spleen distinctly enlarged, the gallbladder apparently normal, the bile ducts not dilated, and an absence of stones. A small piece of liver was excised. The jaundice gradually decreased postoperatively until, four months after onset, icterus could not be detected in the skin or in the sclerae. It reappeared on two later occasions for several days in relation to gastric distress. Six months after the initial complaints, the patient was symptomatically well and showed no jaundice in skin or sclerae; the liver was not palpable; the bromsulphalein test showed no retention of dye after thirty minutes; the van den Bergh test showed 1.5 units.

The excised piece of liver measured 1 by 0.8 by 0.3 cm. Microscopic examination revealed in a small area a fairly well preserved architecture. In the remaining portion of the specimen normal structure was absent; the lobules appeared collapsed. The histologic picture showed different stages of regressive change, restricted almost solely to the hepatic parenchyma. In the still preserved lobules, the cells demonstrated varying degrees of degeneration, as evidenced by swelling, poor staining properties, coarse granules, vacuoles and indistinct cell outlines. Nuclei in degenerating cells showed different phases of karyolysis. In certain cells, nuclei were large and extremely dark. Cells with many nuclei were also observed. In some lobules, the cells in the central zone were necrotic, often

containing yellowish-green pigment. The Kupffer cells were somewhat swollen. Scattered cellular debris and nuclear particles were present. The portobiliary spaces showed an apparent increase of small bile ducts and capillaries, with infiltration by leukocytes, occasional plasma and eosinophil cells, and large oval cells possessing pale, spindle-shaped nuclei. Collapsed lobules were irregular in shape and size. Hepatic columns could not be followed distinctly. Sinusoids were dilated and filled with blood. Scattered in the remaining reticulum were many leukocytes, eosinophils and oval cells with dark round and kidney-shaped nuclei. No mitoses and no definite proliferation of connective tissue could be made out. The large bile ducts contained no exudate or desquamated epithelium. There was no inflammatory reaction in their walls. No bile thrombi were noted.

The histologic changes were explained on the basis of a toxic dystrophy of the liver of a mild but slowly progressive and intermittent character. Comparisons were made with the findings observed in so-called catarrhal jaundice (Eppinger, Bauer, Klemperer) and in subacute toxic atrophy. In view of the lack of demonstrable evidence of syphilis, the recent nature of the hepatic changes and the length of time following administration of the drug, it was doubted whether arsphenamine alone could be the principal causative factor.

#### SUPRARENAL HEMORRHAGE IN ADULTS. RAYMOND S. ROSEDALE.

Suprarenal hemorrhages in adults are fairly well recognized (Seligman, Kraus, Goldzieher, Dietrich and Siegmund). The unilateral type of hemorrhage occurs less frequently than the bilateral, with a clinical picture not dissimilar. The massive unilateral hemorrhage as a primary cause of death is comparatively rare. In such cases, death probably results from extensive hemorrhagic infiltration of the perirenal and retroperitoneal tissues, and not from suprarenal insufficiency, because even in the involved gland there is often found fairly well preserved parenchyma. Death must then be interpreted on the basis of a shocklike result, such as occurs in any extensive retroperitoneal hemorrhage.

CASE 1.—A white man, aged 36, a chauffeur, while loading and transporting 2 tons of coal in a wheelbarrow, suddenly experienced sharp pain in the right costo-vertebral angle, causing him to rest. This discomfort persisted and was followed six hours later by epigastric pain and vomiting. When he was admitted to the Buffalo City Hospital, the upper part of the abdomen was tender and rigid. The leukocyte count was 19,000, 79 per cent being polymorphonuclears. The temperature was 100 F. The blood pressure slowly fell. Because of the progressive shock with severe abdominal pain and vomiting, acute hemorrhagic pancreatitis was suspected, and a laparotomy was performed. Death occurred seventy-six hours after the onset of pain.

At autopsy, the right suprarenal gland was found involved by an extensive hemorrhage. This had destroyed nearly the entire cortex, leaving only thin remnants, and had infiltrated the retroperitoneal tissues downward as far as the pelvic brim, over the pancreas and to the left as far as the hilus of the spleen. There was a soft apparently recent thrombus in the right suprarenal vein.

The histologic picture showed only the changes reactive to severe hemorrhage, such as infiltration by leukocytes. There was necrosis of some of the cortical cells, but, on the whole, cortical and medullary parenchyma was well preserved. Stain for bacteria proved negative.

There were no signs of a primary inflammation in the suprarenal gland itself, nor any thrombophlebitis or thrombosis in the smaller veins of the medulla. It is felt that the recent thrombosis found in the right suprarenal vein was secondary to the extensive hemorrhage.

The usual causes of suprarenal hemorrhages as mentioned in the literature are lacking in this case. The exact cause is not clear. Kraus and others described a mechanical factor involving traction on the hilus of the suprarenal gland due to prolonged contraction of the musculature of the suprarenal vein. They stated their belief that this may cause a rupture of the suprarenal capillaries with acute extensive hemorrhage, as in apoplexy. Because of the definite history of unusual exertion in a person unaccustomed to hard labor, the question arises whether or not the mechanism described by Kraus might have been an important factor in this case.

CASE 2.—In a white man, aged 24, bilateral suprarenal hemorrhage was found. It was not extensive, and there was no destruction or infiltration of the tissues about the gland. The hemorrhage in this case was explained rather on an infectious basis, as chronic gonorrheal urethritis, prostatitis and dermatitis were present. Here the hemorrhage occurred six hours after the passage of a catheter through a urethral stricture.

Many believe that marked suprarenal hemorrhage may occur in a condition of general infection, in which the capillaries of the suprarenal medulla are subjected to extensive diapedesis of erythrocytes. Although a purulent inflammation of the bladder, prostate and skin was present in the second case, I feel that the so-called infectious theory is not sufficient in order to understand the sudden extensive hemorrhage, as there still must be explained why the bilateral hemorrhage followed rather acutely the forceful and very painful passage of a urethral catheter.

#### MARKED ENLARGEMENT OF THE HEART IN INFANCY DUE TO PARENCHYMATOUS MYOCARDITIS. F. E. KENNY.

Two cases of marked dilatation and hypertrophy of the heart in infancy have been studied. Other instances of such cardiac enlargement have been reported, which were termed idiopathic enlargement. Stoloff, in 1927, completely reviewed this subject. These other cases were so called, because of the obscurity of the etiology and the lack of microscopic changes in the myocardium. A careful study of such published reports has revealed, however, in a few instances, degenerative changes and increased connective tissue in the myocardium. Other cases have been reported which occurred in infants and in which true myocarditis was present. In both cases presented here, I wish to stress the history of previous infection and the presence of parenchymatous degeneration of the myocardium.

CASE 1.—A white girl, aged 12 months, had a history of pneumonia six weeks previous to hospitalization. Fatal illness was ushered in by dyspnea, cough and loss of appetite. Autopsy showed the heart to be markedly dilated and slightly hypertrophied, weighing 85 Gm. No congenital abnormalities were found.

Histologic examination revealed a diffuse parenchymatous degeneration throughout the cardiac muscle. The muscle fibers were swollen; cross and longitudinal striations were absent. The cytoplasm was not well stained and showed in some places granular degeneration. Here and there only the sarcolemma remained, enclosing cellular debris, while in other places, no remnants of the muscle fibers could be found. The nuclei were chiefly eccentric in position, with many variations in size and staining qualities, and with chromatin material scattered throughout the nuclear substance. In the outer layers of the myocardium, beneath the epicardium, were many round cell infiltrates, most of the degenerative process being in the inner layers. Fibrous tissue did not seem to be increased in amount.

CASE 2.—A white boy, aged 6 months, had been perfectly well until 3 months of age, and then had suffered from stomatitis and attacks of dyspnea and cyanosis. Three months after the first illness, his condition became more serious; he refused feeding, and became dyspneic and cyanotic. Pericarditis with effusion was diagnosed, and the pericardium was tapped, a slight amount of bloody fluid being withdrawn. Death followed shortly after the paracentesis. Autopsy revealed compression atelectasis of the lungs and marked dilatation and moderate hypertrophy of the heart, which weighed 65 Gm. No congenital abnormalities or vascular anomalies were present.

Microscopically, the endocardium was distinctly thickened. The inner zone of the cardiac muscle showed a patchy degeneration of the myocardium, characterized by pale-staining cytoplasm, and in some places, only the sarcolemma remained. The outer zone showed a marked increase in interstitial fibrous tissue. No cellular infiltration was present.

These two cases of apparently toxic myocardial degeneration seem to be directly traceable to previous infection. In the first case, there was a fairly recent inflammatory condition (pneumonia) with the resulting active process in the cardiac muscle; in the second instance, the infection was more remote, the degenerated areas having been partially replaced by fibrous tissue.



## Book Reviews

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**The Intervertebral Discs: Observations on Their Normal and Morbid Anatomy in Relation to Certain Spinal Deformities.** By Ormond A. Beadle. Medical Research Council, Special Report Series, No. 161. Price, 2 shillings, net. Pp. 179. London: His Majesty's Stationery Office, 1931.

The author presents a summary of the work by Schmorl and his students on about seven thousand spines, and his own observations after examining the same material. His own ideas are, however, in harmony with Schmorl's interpretations. The many illustrations have been supplied by Professor Schmorl.

The section on anatomy discusses the nature of the end-surfaces of the adult vertebral bodies and the construction of the intervertebral disks. The part played by the periphery of the annulus in conjunction with the epiphyseal ring is pointed out.

The section on the adolescent spine gives an account of the normal development, and points out how the different appearance of the growing spine may be explained in relation to the adult structures. Close reading will help to clear up the debated subject of vertebral epiphyses in man.

The discussion on malformations of the intervertebral disks discloses the not infrequent, but little known, developmental defects: persistence of chorda tissue, nuclear expansions of the disks and breaks in the cartilage plates.

Next is a review of the degenerative changes of the fibrous structure of the disks. Changes which in other organs would be interpreted as advanced degenerations occur in the intervertebral disks with surprising frequency, and seem to set in comparatively early. Examination of a large series of spines leaves the impression that in the middle decades of life, as well as later, well preserved disks are the exception rather than the rule. It is pointed out that the changes which may lead to complete loss of function are to be regarded as the results of continued excessive functional strain on tissues which, in the course of increasing age, have become lifeless and desiccated and are no longer able to withstand the burden of activity or to react to adaptation and repair.

It is pointed out that the cartilage plates are subject to a series of changes which are partly further developments of the processes in the fibrous portions of the disks, and partly primary. In general, the cartilage plates are the most resistant parts of the disks, and their important function is to protect the spongiosa, which is provided with no end-plate of compact bone, like the long bones, against the pressure of the nucleus and the tension of the fibers of the annulus lamellosus. The cartilage serves to confine the tissue of the disks to its proper limits and to maintain its normal form and turgor. The plates may be injured by direct trauma and by disease causing advancing destruction of the vertebral body, but under such circumstances the plates show evidence of resistance to destruction. The most common change in the cartilaginous plate is the development of lack of resistive power. As long as the plate is intact, the disk may still perform its work with fair efficiency, even though severely injured in its substance.

The next section discusses prolapse of disk tissue into the spongiosa, and points out that as soon as the cartilage plate gives way, a series of most remarkable changes takes place with a great degree of deformity of the disks. When a break in the continuity of the plate occurs, the turgid nuclear substance passes out into the spongiosa. Such a prolapse causes the disk to lose substance and upsets the stress and strain relations within it. The process slowly limits itself by protective reactions, in young persons mostly through the proliferation of cartilage and bone. In adults, the damage is usually more severe, and granulation tissue invades the disks and they may ossify, and the disks are practically destroyed. Thus the mechanical shocks transmitted during hard work from part to part of the spine are no longer absorbed by the elastic cushions of the disks and strike now

against almost bare bony surfaces. In this way great injuries to various parts of the spine are caused and serious deformities arise.

The eighth section deals with lesions of the disks of external origin, particularly from disease or injury of the vertebral bodies. The effects of trauma, slight shocks of normal life, fractures of vertebral bodies and lesions due to disease are discussed.

The ninth section concerns spinal deformity. The various degenerations and injuries to the disks are nearly always found associated with degrees of spinal curvature, but the large and important group of scoliosis must be excluded here. No definite relation between scoliosis and the changes in the disks has been established. On the other hand, the changes are found to be typical and constant phenomena in various other simpler deformities, notably the kyphoses of youth and age and the growth of senile degenerative disabilities known under the term of spondylosis deformans. According to the author, the clearest division that has yet been made of the kyphoses is a group developing in the young, before the close of growth, and a group occurring in middle and later life.

The kyphoses of adolescence form a fairly well defined group, and appear from the twelfth year onward. These kyphoses are distinguished by characteristic prolapses of the disk in the nucleus region in the lower dorsal spine, a lower dorsal kyphosis and a correspondingly highly developed lumbar lordosis. The curvature is due to the vertebral bodies being wedge-shaped—definitely narrower in front than behind. Senile kyphosis is characterized by a curvature, and in the pure cases the intervertebral disks in the greater part of their extent are well preserved, the various forms of senile degeneration are absent, and the cartilage plates are uninjured except at the anterior ends in the kyphotic segment. The kyphosis is different from that of adolescence in that it exists preeminently in the upper or middle dorsal region.

Spondylitis deformans is then discussed. The kyphosis, although almost always present, is not looked on as an integral part of the disease, but rather as an admixture of a coexistent senile kyphosis. Extensive degenerative changes in the intervertebral disks may be considered as the fundamental lesion of spondylitis deformans. It is the typical senile disease of the spine. The sequel, so far as the spine is concerned, is first an increased movability of the vertebrae on one another, owing to the dissolution of the normal disk fibers. Then, when the disks are fibrosed, there sets in a generalized stiffening of the spine which may proceed to a bony ankylosis. It is conceived that during this earlier stage of greater movability, the osteophytes are formed. These osteophytes are present in practically all cases of spondylosis in greater or lesser degree and give the spine a characteristic appearance. They may grow to an enormous size, and contiguous projections may fuse. They do not occur on the posterior surface of the spine, so that narrowing of the spinal canal and consequent nerve changes do not occur.

It is pointed out that when the aging person escapes senile kyphosis and spondylitis deformans he is liable to develop senile osteoporosis of the spine. The site of the lesion is in the spongiosa, and the disks may be well preserved in spite of great age. The intervertebral disks bulge into the atrophic spongiosa above and below.

Mention is made of Bechterew's disease of the spine. It is supposed to be an oostearthritis of the small intervertebral joints which results in a complete but smooth ossification of the anterior longitudinal ligament without osteophytes or bony protuberances of any kind. The whole spine is converted into a hollow tube with a structure resembling that of the ordinary long bones, with, however, only a thin layer of compact bone in front. A varying degree of kyphosis is also present.

It would be difficult to express the debt which the medical profession owes to Dr. Beadle for summarizing so lucidly and so completely the extensive work of Schmorl and his co-workers. At a time when anatomy is considered dead and when pathologic anatomy is said to have exhausted itself, Schmorl turns his attention to a subject of greatest clinical importance and clearly points a way to a

better understanding of spinal structural changes. The monograph is beautifully illustrated and should be in the hands of anatomists, who could profitably introduce some of these problems to the medical student in the osteology courses. Orthopedists as well as surgeons and physicians will be interested in the new knowledge about the intervertebral disks.

**Physiology of Bacteria.** By Otto Rahn, Professor of Bacteriology, Cornell University, Ithaca, N. Y. Cloth. Price, \$6. Pp. 438, with 42 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

Professor Rahn's intention in writing this book was to coordinate the physical and chemical knowledge of the necessary functions of life. Among the numerous processes of living organisms he has singled out four functions as essential to life. These are endogenous catabolism, energy formation, growth and death. The book is limited to a discussion of these, omitting considerations of motility, phosphorescence, tropisms, morphology, reproductive processes and similar functions.

The plan of the book is original and unique. It is not a review of the literature, although its text and twenty pages of bibliography make it useful as a reference book. It contains a careful analysis and mathematical treatment of the published quantitative results of the experiments of many investigators, together with numerous hitherto unpublished experiments of the author. It is highly critical in the analysis of data and theories, but "in order to counterbalance any personal view-point, each chapter and subchapter contains a summary usually separated according to facts and theories."

As the title indicates, most of the material has been drawn from studies of bacteria. Pertinent material from studies of other lower fungi has been used also, particularly the results of many important investigations of the physiology of yeast. The title by no means indicates the limitations of the scope of the book. The author clearly shows how wide are the ranges of bacteriologic processes and how advantageous these organisms are for investigations in cellular physiology. There is increasing agreement with the opinion of the author that general physiology has much to learn from the physiology of the bacteria, and that the principles set forth in this book reach beyond the domain of bacteriology and apply to biology generally.

The first section of eleven pages is a discussion of endogenous catabolism, the deterioration of cell compounds within the cell. A distinction is made between the autolytic digestion of dead cells and the products arising from the constant deterioration of protoplasmic parts of the cells due to the instability of enzymes and other compounds.

The second section gives an account of the sources of energy for the cell and deals chiefly with fermentation. Fermentation is broadly defined as "the chemical decomposition of compounds within the cell for the purpose of liberating energy." This includes all the intracellular decomposition processes, organic and inorganic, aerobic and anaerobic, with and without gas, of fats, carbohydrates, proteins and other compounds which yield energy. Quantitative data on energy exchange and equations for many types of fermentation are set forth in appropriate groups. The measurements of energy exchanges show that a considerable amount of energy is required to produce the cells of yeast and bacteria from foodstuffs and for the maintenance of energy potentials in the living cell to prevent detrimental reactions. The author adopts the opinion that most of the reactions of fermentation can be explained best by the assumption that the enzymes of the cell activate certain hydrogen atoms in the substrate. These then combine with a hydrogen acceptor, which may combine with another part of the same molecule, with another organic or inorganic molecule or with activated oxygen. The chapters following this section present a critical analysis of the rôle of oxygen in all fermentations.

The third section, on "Growth," discusses chiefly the function of the construction of complex molecules from less complex and the resultant total increase of the living substance of the cell. Processes of growth are separated from fermentation as sharply as possible in the study of a function which is ultimately dependent

on fermentative energy exchange. The author finds that fermentation and respiration are brought about by catalysts of one kind (enzymes), and that multiplication is under the control of catalysts of another kind (genes). Visible morphologic formations are not discussed in this section, but the author has much of interest to say about the probable molecular configurations and arrangements in the living cellular substance.

Data on the death rates of bacteria under a variety of conditions are assembled and analyzed in the fourth section of the book. This contains much valuable information about the action of disinfectants. Through his analysis of the logarithmic order of death of bacteria, the author recapitulates and develops his ideas of the mechanism of death as a continuous chemical process which becomes irreversible when one essential molecule becomes decomposed. "If the order of death is computed under the assumption that the destruction of one certain molecule in the cell is sufficient to prevent growth, the resulting order of death corresponds to that observed with bacteria of uniform age."

Although the book is intentionally restricted to the consideration of the four functions selected by the author as fundamental in the physiology of bacteria, the ramifications of the discussions lead to the examination of many other partial functions. It is not an exposition of the whole of bacterial physiology, but does cover a large part of the field. It is evident that information obtainable by experiment is needed on many subjects included in the sphere of this book. The book seems certain to influence future researches, both through its indication of problems requiring investigation and through investigations it will provoke to test theories and conclusions supported by the author but not accepted by others.

The thoughtful and broad point of view of the author and his mastery of the material give the book an original and individual quality. The reviewer was deeply interested and stimulated by reading it. The publisher is to be thanked for his part in making the book available.

**Handbuch der Blutgruppenkunde.** Bearbeitet von Dr. H. Bürkle-de la Camp, Privatdozent, Oberarzt der chirurgischen Universitätsklinik, Munich; Dr. M. Hesch, Assistent am anthropologisch-ethnologischen Forschungsinstitut der Universität Leipzig; Dr. G. Raestrup, o. Professor, Direktor des Institutes für gerichtliche Medizin der Universität, Frankfurt a.M.; Dr. E. D. Schött, Facharzt für Haut- und Geschlechtskrankheiten; Dr. P. Steffan, Marinegeneraloberarzt, Chefarzt des Marine-Lazarets, Wilhelmshaven; Dr. O. Thomsen, o. Professor, Direktor des Instituts für allgemeine Pathologie, Kopenhagen; I. S. Wellisch, Senatsrat, Wien. Herausgegeben von Dr. Paul Steffan, Marinegeneraloberarzt und Chefarzt des Marinelazarets Wilhelmshaven. Price, 48 marks. Pp. 669, with 125 illustrations and 3 charts. Munich: J. F. Lehmann, 1932.

This handbook on blood grouping consists of 9 parts and 2 appendixes. In the first part Michael Hesch of the anthropologic-ethnologic research institute of the University of Leipzig traces the unbroken course of progress in blood grouping from 1901, when Landsteiner described the existence of human blood groups. The second part, which deals with the serology of blood grouping in man and animals, is written by Oluf Thomsen, professor of general pathology in the University of Copenhagen, who also wrote part 4, on the relations between the blood groups and other hereditary characters, but particularly morbid conditions of various kinds. The inheritance of the factors of blood grouping is discussed in part 3 by Siegmund Wellisch, engineer and Senatsrat in Vienna, who is an active investigator in this field. The practical use of blood grouping in transfusion and transplantation is presented in detail, clinical and operative, by Bürkle-de la Camp of the surgical clinic of the University of Munich. Perhaps more attention should have been given in this section to the actual, practical problems of selecting and controlling donors under various conditions. Part 6, by G. Raestrup, director of the medicolegal institute of the University of Frankfurt a.M., is devoted to blood grouping in legal medicine. Paul Steffan, the editor, Marinegeneraloberarzt in Wilhelmshaven, wrote part 7, on the distribution of the blood

groups in the various human races with reference to its bearing on racial origins. Part 8, the technic of blood grouping, is by Dr. E. D. Schött of Stockholm. These parts or sections constitute separate comprehensive monographs by authoritative writers on the main fields of blood grouping. Part 9, by Michael Hesch, contains the bibliography of blood grouping to 1931; 2,979 titles are given, arranged alphabetically by authors' names and covering 98 pages. The appendixes by S. Wellisch contain tables of squares and square roots, in order to facilitate the calculations of indexes. There is a complete subject index at the end.

The book summarizes conveniently and thoroughly what is known about blood grouping and its application for practical and scientific purposes. The component parts are well balanced and coordinated. It is a great central storehouse of information. This handbook and Lattes' "Individuality of the Blood," recently translated into English by L. W. Howard Bertie (Oxford University Press), fully meet the present need of investigation and practical work for comprehensive presentations of blood grouping.

**Primary Carcinoma of the Lung: Bronchiogenic Cancer. A Clinical and Pathological Study. In Two Parts.** By B. M. Fried, M.D., Peter Bent Brigham Hospital, Boston. Price, \$5. Pp. 247, with 95 illustrations. Baltimore: Williams & Wilkins Company, 1932.

This addition to "Medicine Monographs," presented as a clinicopathologic review, serves once more to bring into prominence the increasing importance of cancer of the bronchi as a disease entity. The author considers the various histogenetic theories in detail, and presents convincing arguments in favor of the view that all pulmonary carcinomas probably arise from the basal cells of the bronchial mucosa, and that the advanced differentiation of some tumors is the result of a form of metaplasia of these cells.

Besides the article as it originally appeared in "Medicine," the book contains the details of all the case studies on which the report is based. This additional material comprises 102 of the 234 pages of reading matter, and is divided into two groups, the one of "typical" instances, in which the correct diagnosis might possibly have been suggested by the bronchopulmonary symptoms, and the other of an "atypical" group, in which the disease was characterized by symptoms other than those calling attention to the lungs. In the latter group, the records of tumors the first symptoms of which were due to cerebral metastases are particularly illuminating. Many such patients had undergone operations for removal of the tumor in the brain. The subsequent history of these patients seems to indicate that such a procedure is often valuable as a palliative measure, and that the knowledge that an intracranial growth is secondary to a bronchial carcinoma should not, in itself, contraindicate an attempt to remove the metastasis.

The value of the work is greatly enhanced by an abundance of excellently reproduced illustrations of gross and microscopic lesions and roentgenograms. The format is attractive, although typographic errors appear with the usual frequency in the volumes of this series. Appended are a list of references and an index.

The book should be especially helpful to physicians, who will find in the descriptions of some of the more bizarre manifestations of the disease many suggestions of aid in diagnosis.

**Fungous Diseases: A Clinico-Mycological Text.** By Harry P. Jacobson, M.D., Attending Dermatologist and Member of the Malignancy Board, Los Angeles County General Hospital. With an Introduction by J. Frank Schamberg, M.D., Professor of Dermatology and Syphilology, Graduate School of Medicine, University of Pennsylvania, and Howard Morrow, M.D., Clinical Professor of Dermatology, University of California Medical School. Price, \$5.50. Pp. 290, with 153 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

This treatise brings up to date a summary in textbook form of the clinical and scientific aspects of the important mycotic diseases affecting the human species.

It is a practical reference book, and a timely one, on classification, diagnosis, prognosis, technic and therapy, and is arranged for easy reference. The book offers to the practitioner and the medical student a comprehensive discussion of this subject, a feature which alone should make it welcome. The book is practically complete in the light of present knowledge, and is well written. All pertinent literature is included in the bibliography of each subject, and credit is given where it is due. The author has borrowed freely of photographs, and, for the most part, the illustrations are good. It is noticeable that the recent findings of mycotic nature in *perlèche* of the types seen both in children and in adults are not included in this book.

The contents are divided into ten chapters, but into three major divisions, a logical grouping. These divisions are: (A) primary cutaneous mycoses with (usually) no definite systemic involvement (this includes the dermatomycoses, comprising all forms of ringworm and favus); (B) primary cutaneous and mucous membrane infections with frequent systemic involvement (moniliasis, mycetoma, sporotrichosis, blastomycosis, actinomycosis and coccidioides), and (C) primary systemic infections with occasional instances of cutaneous or mucous membrane involvement, including torulosis and aspergillosis.

**Clinical Endocrinology of the Female.** By Charles Mazer, M.D., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, University of Pennsylvania; Gynecologist to Mount Sinai and Northern Liberties Hospitals, Philadelphia, and Leopold Goldstein, M.D., Demonstrator of Obstetrics, Jefferson Medical College; Assistant Gynecologist to Mount Sinai Hospital. Price, \$6, net. Pp. 518, with 117 illustrations. Philadelphia: W. B. Saunders Company, 1932.

The purpose of this book is to review the knowledge of the relations between the female generative functions and the endocrine secretions, with particular reference to the application of that knowledge in clinical diagnosis and treatment. The book opens with an introductory section followed by two comparatively long chapters on the ovary and its hormones and on the pituitary gland and its hormones. Then come briefer considerations of the thyroid and other endocrine glands and of endocrine relationships. The clinical value of the tests for ovarian and pituitary sex hormones in the blood is reviewed, with descriptions of the technical details. Chapters 8 to 11 deal with menstruation, its disorders and their treatment with glandular products in particular. There are chapters on functional sterility, endocrine regulation of pregnancy and labor, the hormone tests for pregnancy, lactation, the rôle of the hormones in the menopause, ovarian transplantation as a therapeutic measure and endocrine obesity. The book closes with a list of 649 references, an author index and a subject index. The chapter on the hormone tests for pregnancy will be of value to those who make such tests. The influence of the luteal hormone on the endometrium will interest the pathologist who studies uterine scrapings microscopically. The developments in the endocrinology of the female sex invest the pathologic anatomy and histology of the female reproductive organs with a new interest. The treatment of the various topics is sensible and conservative. There is no tendency to exaggeration or mysticism. The style is clear; the illustrations are commendable. In all writings on clinical endocrinology special care should be given to the choice of names to designate various endocrine products, in order to avoid confusion.

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A HANDBOOK OF EXPERIMENTAL PATHOLOGY. By George Wagoner, M.D., Associate in Pathology, and R. Philip Custer, M.D., Associate in Research Pathology, University of Pennsylvania. Price, \$4. Pp. 160. Springfield, Ill.: Charles C. Thomas, 1932.

POLIOMYELITIS: A SURVEY MADE POSSIBLE BY A GRANT FROM THE INTERNATIONAL COMMITTEE FOR THE STUDY OF INFANTILE PARALYSIS ORGANIZED BY JEREMIAH MILBANK. Price, \$6. Pp. 562. Baltimore: The Williams & Wilkins Company, 1932.

POSSIBILITIES AND NEED FOR DEVELOPMENT OF LEGAL MEDICINE IN THE UNITED STATES. WITH A SUPPLEMENT ON UNIVERSITY DEPARTMENTS IN THE FIELD OF CRIMINOLOGY. Bulletin No. 87. Prepared for the National Research Council Committee on Medicolegal Problems by Oscar T. Schultz. Price, \$1.50. Pp. 135. Washington, D. C.: The National Research Council of the National Academy of Sciences, 1932.

SEKTIONSTECHNIK. Von Professor Dr. Robert Rössle, Direktor des Pathologischen Instituts der Universität Berlin. Price, 2.20 marks. Pp. 50, mit 7 Abbildungen. Berlin: Julius Springer, 1932.

FACIAL GROWTH IN CHILDREN WITH SPECIAL REFERENCE TO DENTITION. Part I by Corisande Smyth. Part II by Matthew Young. Medical Research Council Special Report Series No. 171. Price, 1s. 6d., net. Pp. 83. London: His Majesty's Stationery Office, 1932.

MEDICAL USES OF RADIUM: SUMMARY OF REPORTS FROM RESEARCH CENTRES FOR 1931. Medical Research Council Special Report Series No. 174. Price, 1s. 3d., net. Pp. 59. London: His Majesty's Stationery Office, 1932.

TENTH SCIENTIFIC REPORT ON THE INVESTIGATIONS OF THE IMPERIAL CANCER RESEARCH FUND. Under the direction of the Royal College of Surgeons of England. Published by the Authority of the Executive Committee. Price, 30s. Pp. 203, with 55 plates. London: Taylor and Francis, 1932.

TYPHO EXANTHEMATICO DE SAO PAULO. By José de Toledo Piza, J. R. Meyer and Luis Salles Gomes. Pp. 156, with 52 figures. Sao Paulo: Sociedade Impressora Paulista, 1932.

DER CORONARKREISLAUF PHYSIOLOGIE, PATHOLOGIE, THERAPIE. Von Dr. Max Hochrein, Professor an der Universität Leipzig. Pp. 227, mit 54 Abbildungen. Price, 24 marks. Berlin: Julius Springer, 1932.

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